Current and new concepts in the diagnosis and management of diabetic macular oedema

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Abstract

Diabetic macular oedema, which can cause rapid visual deterioration, may not have early warning signs at times. Assessment of diabetic retinal complications is made chiefly by clinical examination combined with optical coherence tomography (OCT) and fundus fluoroscein angiography (FFA). However, assessment usually does not occur until the late stages of diabetic retinopathy (DR), and, as retinal neurologic changes precede clinical changes, as tested in this thesis, by the time clinical assessment is performed, much of the functional visual loss has already occurred. More robust diagnostic modalities are required to detect progression of retinopathy in the early stages, before irreversible damage has already happened, and advances in the treatment of diabetic macular oedema is imperative as the current standard treatment in the form of laser photocoagulation is ineffective in improving the vision as authenticated in the following chapters.

In this thesis, both treatment and diagnostic strategies of diabetic macular oedema (DMO) are investigated. Although laser photocoagulation is effective in short term in treating diabetic macular oedema, its mechanism of action is unknown; is associated with considerable collateral damage; and long term visual prognosis is meagre at a mean change in visual acuity at 5 years of -5.23. The 3-year outcome was also inferior to the clinical trial results with more people gaining vision (≥ 15 letter gain) in the diabetic retinopathy clinical research network (DRCRN) group compared to this cohort (26% versus 9%). Furthermore, three times more patients lost vision (> 15 letter loss) in the real-life setting of this cohort compared to the clinical trial results of the DRCRN group (27% versus 8%, respectively). Therefore, improved preventative and treatment

modalities are essential to prevent progression in the early stages and to improve functional vision in late stages.

In an attempt to look for new treatment strategies, we hypothesized that retinal oxygenation by inhibition of dark adaptation in the rod photoreceptor, could possibly inhibit progression of diabetic maculopathy. Illuminated-mask treatment of individuals with early diabetic maculopathy revealed encouraging results that point to an inexpensive and non-invasive therapy. Whilst 19 out of 34 study eyes with cysts at the beginning of the trial improved, 11 out of 30 fellow eyes with no demonstrable cysts at the onset developed cystic macular changes towards the end of 6 month trial.

In the final chapters the correlation of visual functions with anatomic appearance were examined. The results of functional assessments, including visual acuity, colour contrast sensitivity, and microperimetry, had variable relation to structural changes at the macula with OCT. Therefore, an urgent need remains for the development of reliable diagnostic and preventative tools for the early assessment and treatment of visual function defects related to diabetic macular oedema.

Acknowledgements

It is a great pleasure to thank all of those who made this thesis possible. I would like to gratefully and sincerely thank Miss Sobha Sivaprasad (SS), for her supervising, guidance, understanding, patience and support all through my project. Her mentorship has been paramount in achieving this task. She has made available her support in many ways as in discussing and drafting out the projects, planning and implementation. She encouraged me all throughout to think and come up with new ideas, develop various essential research skills. I am very much indebted to all my former colleagues from the research department of King's college hospital who helped me all through my research work, particularly Liz, Mathew and Richards in assisting with the technical aspect of the research work, and Irene for her research coordination, and my other colleagues for their understanding whilst working under pressure. I owe my sincere and earnest thankfulness to Dr Tracey Bailey, my other supervisor from Cranfield University, for her encouragement to complete the thesis, keep targets and dead lines, which stimulated me in finishing the projects. Many thanks for the extra patience you have shown and looked closely at my written work all through the research project and helped with English style and grammar, correcting both and offering suggestions for improvement.

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I dedicate this thesis to my wife Aparna, my beautiful daughters Pranathi (Chibbi) and Charvi (Chikki) for their unconditional love and understanding all through the process of this research, thesis submission and beyond. I thank my parents Gopal and Ramani, in having faith in me in whatever I pursued with and for all their blessings.

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Abbreviations and symbols

AGE	-	Advanced glycation end products
ARMD	-	Age related macular degeneration
AUC	-	Area under curve
BAX	-	BCL 2 associated X protein
BCL 2	-	B cell lymphoma 2
BMI	-	Body mass index
BRB	-	Blood retinal barrier
cGMP	-	Cyclic guanosil mono-phosphate
CNVM	-	Choroidal neovascular membrane
CS	-	Contrast sensitivity
CST	-	Central Subfield Thickness
CI	-	Confidence interval
DM	-	Diabetes mellitus
DAG	-	Diacyl glycerol
DMO	-	Diabetic macular oedema
DNA	-	Deoxyribo nucleic acid
DPP	-	Di-peptidyl peptidase
DR	-	Diabetic retinopathy
ERG	-	Electro retinogram
ETDRS	-	Early Treatment Diabetic Retinopathy Study
GC	-	Guanyl cyclase
GDNF	-	Glial cell derived neurotrophic factor
GDP	-	Guanosil di-phosphate
GFAP	-	Glial fibrillary acidic protein
GLP	-	Glucagon like peptides
GLP-1	-	Glucagon-like peptide-1
GSH	-	Glutathione
GSSG	-	Oxidized glutathione
GTP	-	Guanine-triphosphate
HbA1C	-	Haemoglobin A1C
HIF	-	Hypoxia inducible factors
ICC	-	Intraclass Correlation Coefficient
IGF	-	Insulin like growth factor
IVTA	-	Intra vitreal triamcinolone
KCH	-	Kings' College Hospital
MP1	-	Microperimetry-1
NFL	-	Nerve fibre layer

NF-Kβ	-	nuclear factor kappa-light-chain-enhancer of activated B cells
PDE	-	Phosphodiesterase
PDR	-	Proliferative diabetic retinopathy
PDGF	-	Platelet derived growth factor
PKC	-	Phospokinase C
RGC	-	Retinal ganglion cells
ROC	-	Receiver operated characteristics
ROS	-	Reactive oxygen species
RPE	-	Retinal pigment epithelium
SD	-	Standard deviation
SJ	-	Author, Dr Sreedhar jyothi
SOD	-	Super oxide dismutase
SS	-	Supervisor, Miss Sobha Sivaprasad
SMC	-	Smooth muscle cells
TGF-β	-	transforming growth factor beta
TNF	-	Tumour necrosis factor
UKPDS	-	United Kingdom Prospective Diabetic Study
VA	-	Visual acuity
VEGF	-	Vascular endothelial growth factor
VIP	-	Vaso active intestinal peptide
VSMC	-	Vascular smooth muscle cell
ZO	-	Zonula occludens protiens

1: INTRODUCTION

1.1 Epidemiology of diabetes mellitus & related ocular complications

Globally, the prevalence of diabetes mellitus (DM) in adults aged ≥20 years was 4.0% in 1995, 4.5% in 2010 and the number is expected to reach 5.4% by 2030 (Amos AF, 1997; King H, 1998; Wild S, 2004). A more recent study has estimated the prevalence of DM was 6.4% in 2010 and will increase to 7.7% by 2030 (Shaw JE, 2010). These percentages correspond to an increase to 522 million patients with diabetes from the current 366 million (Whiting DR, 2011). Cases of diabetes-related complications are expected to increase rapidly to 20-30% above present levels between 2035 and 2045 (Bagust A, 2002). For the UK, Amos et al. (1997) projected the growth of diabetes at 4.1%. Currently, the UK has more than 2.6 million people with diabetes and estimated to increase up to 4 million by 2025, of which approximately 90% have Type 2 and 10% have Type 1 diabetes (Forouhi NG, 2006; Diabetes UK, 2010).

DM over time can lead to various complications and among which the macro vascular complications include cardio-vascular disease, stroke and peripheral vascular disease and the micro vascular complications of diabetes include neuropathy, nephropathy and diabetic retinopathy (DR). Diabetic retinopathy with its clinical manifestations of Proliferative Diabetic Retinopathy (PDR), Vitreous Haemorrhage (VH), Diabetic Macular Oedema (DMO) and Clinically Significant Macular Oedema (CSMO) is one of the leading causes of blindness in developed countries (Klein BE, 2007; Taylor HR, 2001; Ockrim Z, 2010). Diabetic macular oedema is the leading cause of visual impairment in patients with DR (Resnikoff S, 2004). A recent meta-analysis has put the age standardised global prevalence rates of diabetic eye disease at 35.4% (35.2–35.6) for any DR, 7.24% (7.15–7.33) for PDR, 7.48% (7.39–7.57) for DME, and 11.7%

(11.6–11.8) for VTDR. Extrapolating these percentages to the contemporary world diabetic population at the time of the study there were 92.6 million (91.2– 94.0) adults who had any DR, 17.2 million (16.6–17.7) had PDR, 20.6 million (19.6– 21.6) had DME, and 28.4 million (27.6– 29.2) had VTDR. Some of the other prominent epidemiologic studies quoted the prevalence of DR at the time of diagnosis of Type I diabetes is in between 0 and 3% (Klein R, 1997) and in Type II there is higher evidence of presence of retinopathy at 6.7-30.2% (Kohner EM, 1998). Nearly all patients have some degree of retinopathy after 15-20 years of Type 1 DM and the figure is 60% in Type II diabetics (Aiello LP, 1998). The prevalence of macular oedema (DMO) is found to be proportional to the duration of diabetes. The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) stated that 0% of Type I diabetics showed evidence of DMO with less than 5 years of disease duration compared with 29% after 20 years duration. Similarly in Type II diabetics, DMO was found in 3% of patients with less than 5 years disease duration and 28% after 20 years duration (Klein R, 1984). The 10-year incidence of any DR, DMO, or CSMO in WESDR study was 89.3, 20.1, and 9.2% in Type I diabetics, 79.2, 25.4, and 32.8% in insulin-treated Type II diabetes, and 66.9, 13.9, and 21.4% in noninsulin-treated Type II diabetes (Klein R, 1996). In a subsequent study by the same group, 25-year cumulative rate of progression of DR has been calculated as 83% in Type 1 DM and the 25-year cumulative incidence of diabetic macular oedema (DMO) and clinically significant macular oedema (CSMO) found to be 29% and 17% respectively (Klein R, 2008, 2009). A UK-based study estimated DR prevalence among patients with DM at 40% and sight-threatening retinopathy (STR) at 14% (Raymond NT, 2009) and prevalence of CSMO at 6.4-6.8%. It was estimated in the UK in 2008 that 218,000 people had severe sight loss (blindness), and the numbers

predicted to double by 2050 (Access economics, 2009). Of this number, 8.7% are reported to be owing to diabetic eye disease. Our understanding of the pathophysiologic mechanisms underlying DR and the development of complications is constantly evolving, due to ongoing research (van Dijk HW, 2012; Curtis TM, 2012; Antonetti DA, 2006; Curtis TM, 2009; Ciulla TA, 2003), and various hypotheses have been put forward which will be discussed further in the following sections.

It is well known from the DCCT (Diabetes Control and Complications Trial) that DR and its complications can progress despite strict glycaemic and blood pressure control, and at a certain threshold, its effects become irreversible. This phenomenon is known as "retinopathic momentum" and at this stage any form of intervention will not affect progression (Bailey CC, 1999). This may occur with no visual symptoms, so early screening and prevention of visual loss is imperative (DCCT, 1993)

1.2 Normal Retina

The retina, which literally means 'net', is formed from two parts of the embryonic optic cup: a) Neurosensory retina from the inner wall, b) Retinal pigment epithelium from the outer wall. The inner neuroblastic cells of the neurosensory retina differentiate into ganglion cells, Muller cells and amacrine cells, whilst the outer cells differentiate into rods and cones, bipolar cells and the horizontal cells. The macula is a comparatively dark area of 5.5 mm in diameter, situated at the posterior pole of the eyeball, temporal to optic disc and contributes to the central 10-15° of the visual field (**Figure** 1.1). The fovea is the central depressed part of the macula and is 1.85 mm in diameter and corresponds to the central 5° of the visual field. The foveola forms the central floor of

the fovea. The Fovela avascular zone (FAZ) is located inside the fovea but outside the foveola.

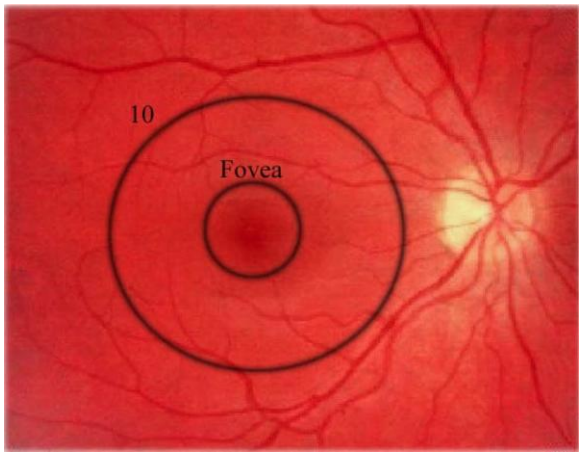

Figure 1.1: Colour photograph of fundus showing macula (outer circle)

The main function of the retina is to catch light via its photoreceptor and pigment epithelial cells. The photoreceptor cells' photopigment molecules absorb the light, causing a change in the photoreceptor's membrane potential. This initiates a series of signals that travel through the neurons of the retina, and into the optic nerve leading to the brain. The neural cells involved in this process are remarkably similar to those of the brain (Litzinger TC, 2002). The microscopic structure of the retina includes three types of cells and their synapses arranged in 10 layers (**Figure** 1.2).

1.2.1 Layers of retina:

Retina is laid out in 10 layers, each with a specific role in the processing the light stimulus and maintaining the integrity of the tissue. The layers from out to in are as given in **Table** 1.1 and **Figure** 1.2.

The RPE (retinal pigment epithelium) forms the outer most layer of retina. It is firmly adherent to the underlying Bruch's membrane (basal lamina of choroid) and is loosely

adherent to the layer of photoreceptors. The potential space between the RPE and sensory retina is called the subretinal space. Layer of rods and cones are considered as neuroepithelium and the end organ for vision. Rods are absent in an area of 300 μm at the centre of fovea; but are present in a large number around the fovea in a 5-6mm ring shaped zone.

Table 1.1: Retinal layers

1) Retinal pigment epithelium (RPE)	6) Inner nuclear layer
2) Photoreceptor layer (Rods and Cones)	7) Inner plexiform layer
3) External limiting membrane	8) Ganglion layer
4) Outer nuclear layer	9) Nerve fibre layer
5) Outer plexiform layer	10) Internal limiting membrane

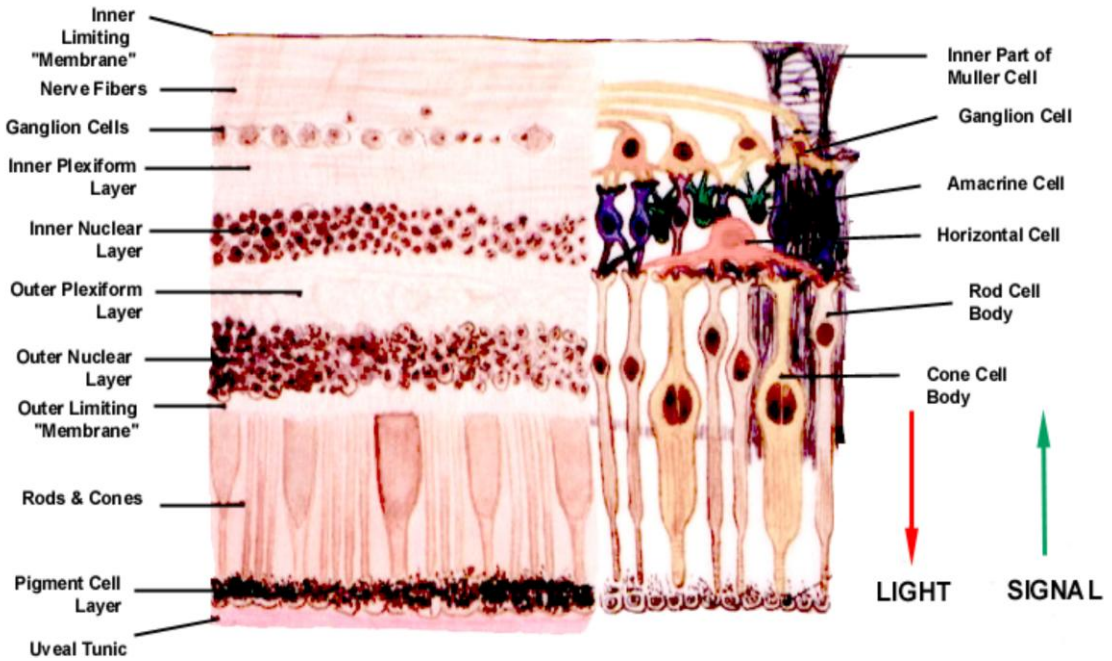

Figure 1.2: Retinal layers and cells (adopted from www.vetmed.vt.edu), May 2013

The external limiting membrane, which appears to pass through the processes of photoreceptors, is formed by junctions (zonulae adherens) between the cell membrane of photoreceptors and Muller cells. The outer nuclear layer is formed by the nuclei of rods and cones. The outer plexiform layer is the synapses between rod and cone pedicles with the dendrites of bipolar cells and processes of horizontal cells. This layer marks the transition between the outer and inner layers. The inner nuclear layer contains the nuclei of bipolar cells, horizontal cells, and the majority of the amacrine cells. The inner nuclear layer is followed by the inner plexiform layer, which essentially consists of synapses between axons of bipolar cells and dendrites of ganglion cells and the processes of amacrine cells. The ganglion cell layer consists of cell bodies and nuclei of ganglion cells. This layer is absent in the region of the foveola. The axons of ganglion cells extend in the opposite direction towards the nerve fibre layer. These cells are responsible for initiating the cascade of events that takes an image projected onto the retina, and converting it from photons to an electrochemical signal capable of being read by the brain.

1.2.2 Cells involved:

Three types of cells comprise the retinal structure (Khurana AK, 2008).

1.2.2.1 Vascular cells:

Pericytes are modified smooth muscle cells (SMCs) that regulate vascular flow by dilating and contracting. Endothelial cells line the inner capillary wall, regulate haemostatic function, and constitute the inner blood retinal barrier (BRB) (**Figure** 1.3).

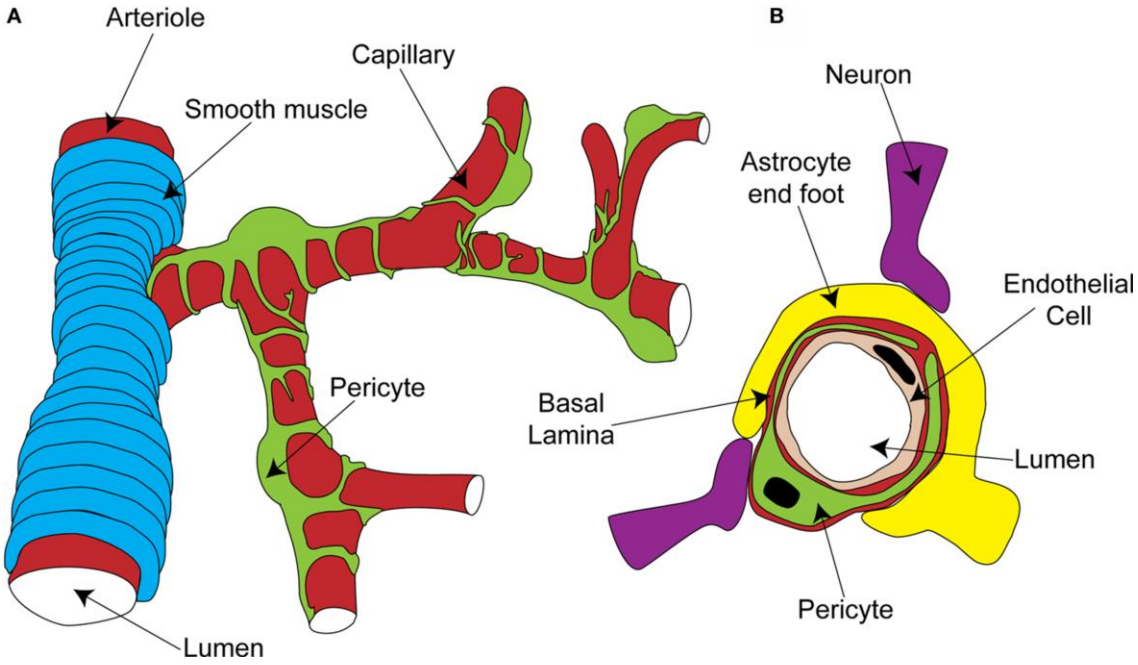

Figure 1.3: Organisation of the capillary neurovascular unit. (A) Rings of smooth muscle encircle arterioles, while pericytes send processes along and around capillaries, without fully covering the vessel. (B) Pericytes are located outside the endothelial cells and are separated from them and the parenchyma by a layer of basal lamina. In the parenchyma, astrocyte end-feet and neuronal terminals are closely associated with the capillary. (Hamilton NB, 2010)

1.2.2.2 Neural cells:

Neural cells of the retina include photoreceptors as well as retinal pigment epithelial (RPE), amacrine, bipolar, and ganglion cells. These cells mediate phototransduction; they convey nerve impulses from the photoreceptor across the synapse to the bipolar and retinal ganglion cells, and to the lateral geniculate nucleus of the brain (Masland RH, 2001).

1.2.2.2a RPE cells:

These form the outer most layer of the neural retina and provide supportive functions to the photoreceptors. (Strauss O, 2005).

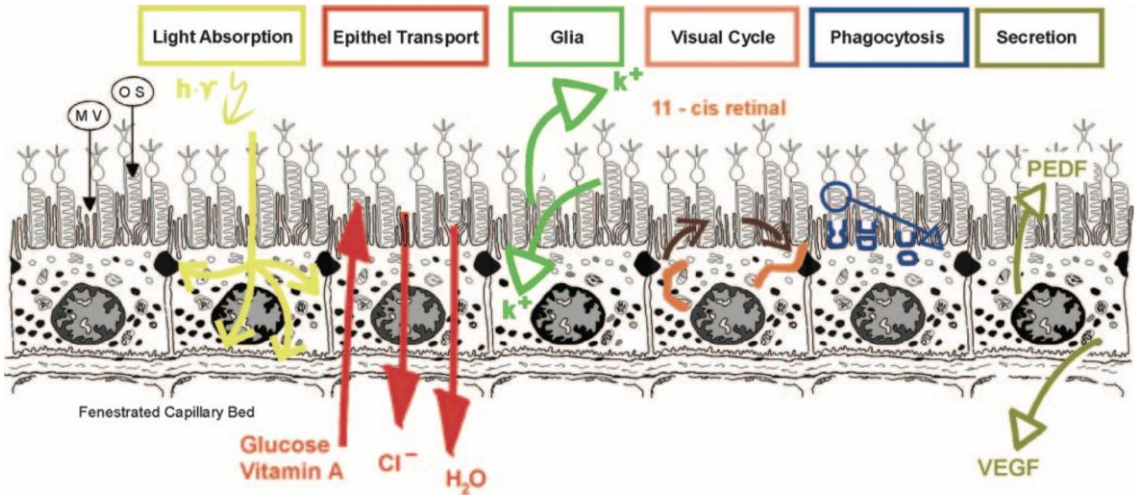

Figure 1.4: The Retinal Pigment Epithelium in Visual Function. PEDF (pigment epithelium derived growth factor), VEGF (vascular endothelial growth factor) (Strauss O, 2005)

The RPE functions in brief are:

a) Important role in photoreceptor renewal and recycling of vitamin A

b) Form the outer blood-retinal barrier and actively pumps water and ions from sub retinal space.

c) Supply inter photoreceptor matrix and help with retinal cell adhesion

d)Transports nutrients and metabolites through blood-retinal barrier and elaborates extra cellular matrix.

e) Regulate and transports ions, water, growth factors and nutrients to the outer segments of the photoreceptors.

f) Phagocytose outer tips of photoreceptors and recycle them

g) Provides mechanical support to the layer of photoreceptors

h) Help in manufacturing photopigment

i) Reduce scatter of light to the photoreceptors and shield them from excessive light exposure by using the Melanin pigment.

1.2.2.2b Photoreceptors (Figure 1.5)**:**

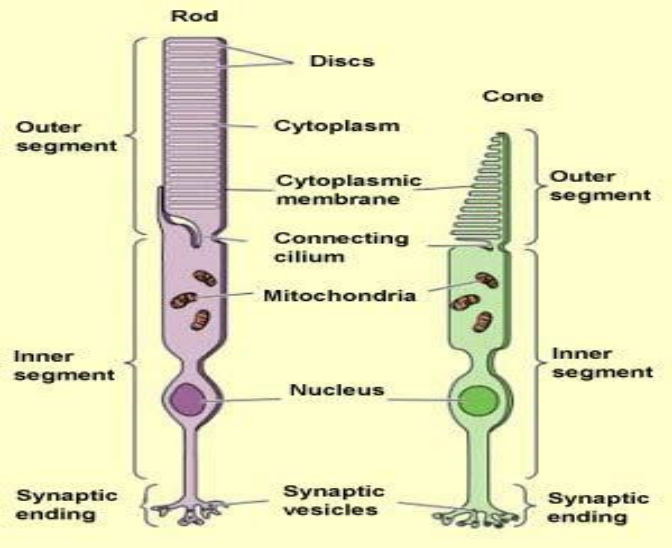

Figure 1.5: Retinal photoreceptors. Shown are inner segments with synaptic endings, body with most mitochondria, and outer segment disks where photo transduction occurs. (Picture adapted from "www.thebrain.mcgill.ca", Aug 2011)

The adult human retina has approximately 130 million photoreceptors of which approximately 120 million are rods and rest is cones (Khurana AK, 2008). Rods are long cylindrical structures and are highly sensitive to light and send signals of grey shades to the brain. Cones are thicker and shorter compared to rods, and send signals of fine details and colour to the brain. The photoreceptors capture light and convert it into electrical signals by a process called phototransduction and is transmitted to brain,

which is perceived as light images. The critical element in this process of phototransduction is the photopigment within the photoreceptors. Opsin is the light sensitive protein within the photoreceptors, which binds with vitamin A, and the complex is called rhodopsin in rods. When light photons strike, this bonding is broken and disrupts the electrical field within the photoreceptor, which initiates the electrical impulse. Where as the cones possess three different types of opsins, which individually bind to vitamin A and are responsible for perception of three primary colours: red, blue or yellow. Cones are not as sensitive as rods for low intense light and therefore need very specific wavelength of light to initiate the electrical impulse. Cones possess complex network of postsynaptic connections in contrary to rods. The bipolar cells, which have synaptic connections with photoreceptors respond differently to excited cones and there by create "ON" and "OFF" signals (Nawy S, 1991). Phototransduction (**Figure** 1.6) in the photoreceptors involves absorption of photon energy by the 11-cis-retinal molecule of opsin molecules, which are G-protein coupled receptors in the photoreceptor cell membrane. Absorption of photon by 11-cis-retinal leads to formation of all-trans-retinal, which stimulates the attached opsin, transducin, to bond with guanine-triphosphate (GTP). The GTP binding causes the alpha-subunit of the transducin to translocate to an enzyme called phosphodiesterase (PDE) located on the membrane, where it binds to the inhibitory gamma subunit of PDE. This binding event decreases the inhibitory effect of the gamma PDE subunit, causing PDE to start actively hydrolysing cyclic guanine-monophosphate (cGMP). Channels in the photoreceptor membrane bind cGMP and open, releasing sodium (Na^{+}) and calcium ions (Ca^{2+}) into the cell. Loss of cGMP by PDE activity causes closure of these channels, which in turn causes the whole cell to hyperpolarise and induce a signal (Yau KW, 1994).

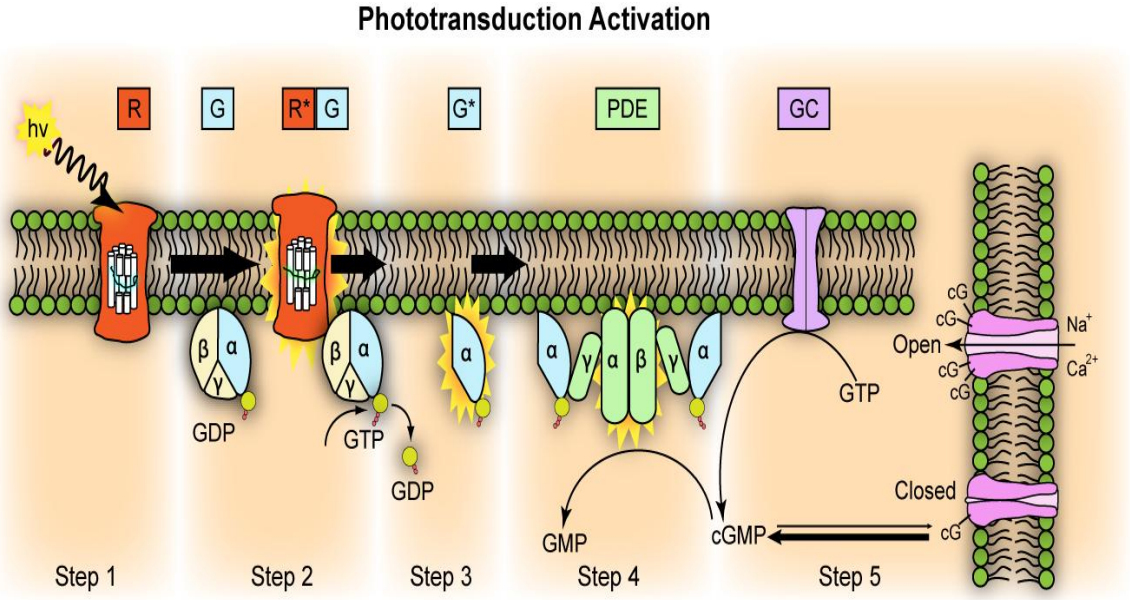

Figure 1.6: Molecular steps in photoactivation. Depicted is an outer membrane disk in a rod. Step 1: Incident photon (hv) is absorbed and activates a rhodopsin by conformational change in the disk membrane to R*. Step 2: R* makes repeated contacts with transducin molecules, catalyzing activation of R* to G* by release of bound GDP in exchange for cytoplasmic GTP. Step 3: G* binds the inhibitory γ subunits of PDE, thereby activating its α and β subunits. Step 4: Activated PDE hydrolyzes cGMP. Step 5: Guanylyl cyclase (GC) synthesizes cGMP, the second messenger in the phototransduction cascade. Reduced levels of cytosolic cGMP cause cyclic nucleotide gated channels to close; this closure prevents further influx of Na+ and Ca2+. Figure adapted from Leskov IB, 2000.

1.2.2.2c Bipolar cells:

Bipolar cells (**Figure** 1.7) are neuronal cells in the inner nuclear layer connecting outer plexiform layer to inner retinal layers. They have a central body, dendrites and axons (Masland RH, 2001). Dendrites receive information from photoreceptors and horizontal

cells and pass it on to ganglion and amacrine cells through axons in the inner plexiform layer. The bipolar cells could be designated either as rod or cone bipolar cells based on their respective connections, and as “ON” or “OFF” cells based on their response to glutamate released by photoreceptors (Nawy S, 1991).

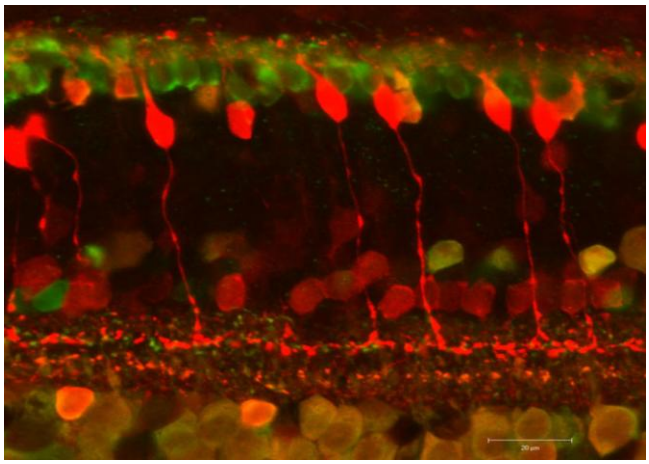

Figure 1.7: Bipolar cells. Picture adapted from (http://www.retinalmicroscopy.com)

1.2.2.2d Horizontal cells (Figure 1.8**):**

Horizontal cells form part of lateral neurons along with amacrine cells. They are laterally interconnecting neurons present in the outer plexiform layer and help with integrating the information received from multiple photoreceptor cells (Masland RH, 2001). They help in adjusting the eyes to see well under bright and dim conditions and increase contrast. They help in increased visual acuity by enhancing lateral inhibition.

1.2.2.2e Amacrine cells (Figure 1.9**)**

Amacrine cells are interneurons in the inner nuclear layer and synapses in the inner plexiform layer with bipolar and ganglion cells, and influence retinal signal processing (Masland RH, 2001). They are responsible for complex processing of the retinal image brightness, contrast and detecting motion.

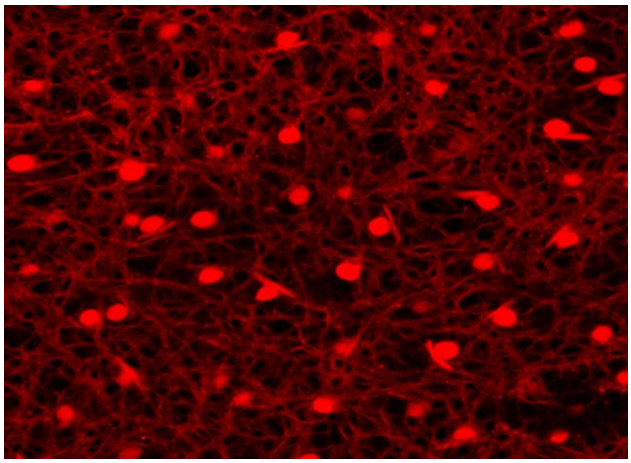

Figure 1.8: Horizontal cells. Picture adapted from (http://www.retinalmicroscopy.com)

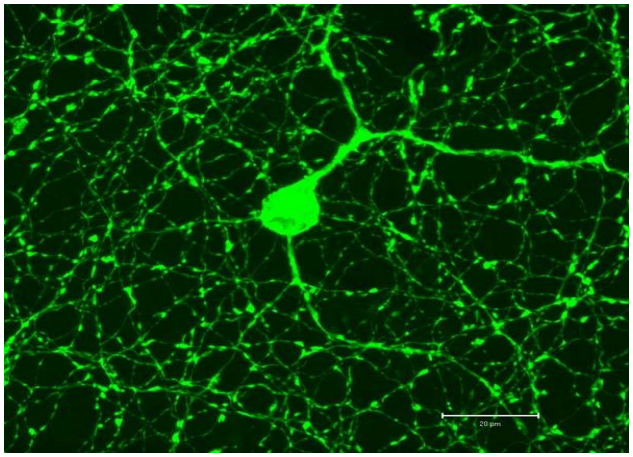

Figure 1.9: Amacrine cells. Picture adapted from (http://www.retinalmicroscopy.com)

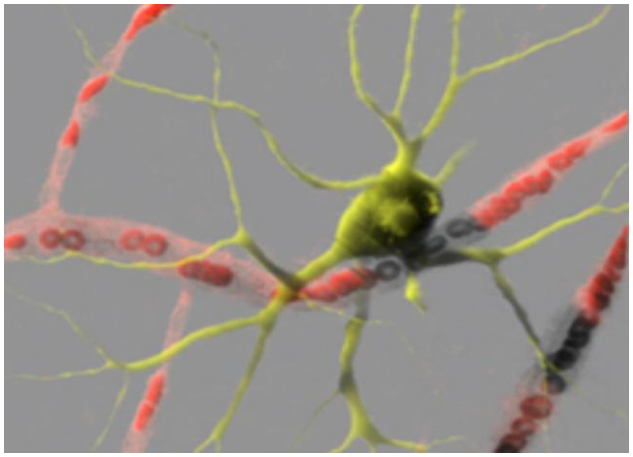

Figure 1.10: Ganglion cells. Picture adapted from www.ucl.ac.uk

1.2.2.2f Ganglion cells (Figure 1.10)

Ganglion cells are a type of neuronal cells, which collect the visual information from bipolar and amacrine cells through their dendrites and the final output reaches the brain through their axons. Ganglion cells vary significantly depending on their projections, functions, based on which different types of gangion cells may be defined: "W" cells are excited by rods and function to perceive directional movement. The "X" cells are responsible for colour vision. The axons of the ganglion cells of the retina converge, forming the optic nerve. The "Y" cells are largest of all and assist with perception of changes in light intensity and rapid movements of the image. Ganglion cells have action potentials unlike other integrator neurons and changes in light intensity and shifting of images over the field of vision cause a change in the firing rate in ganglion cells (Masland RH, 2001). These "ON" and "OFF" responses, which take the form of changes of frequency of firing, are interpreted by brain as final output signals.

1.2.2.3 Glial cells (Figure 1.11):

Three types of glial cells are found in the retina: Muller cells, microglia, and astrocytes of which Muller cells are the major component. The interaction of these cell types is crucial for the functional integrity of retinal metabolism.

Macroglia are support cells that regulate the retinal metabolism and modulate the function of neurons and blood vessels (Abbott NJ, 1992). Muller cells form the architectural support; provide metabolic support and homeostatic regulation. They are located in the inner nuclear layer and project their processes from internal limiting membrane to the outer limiting membrane where junctional complexes with bases of photoreceptors are formed.

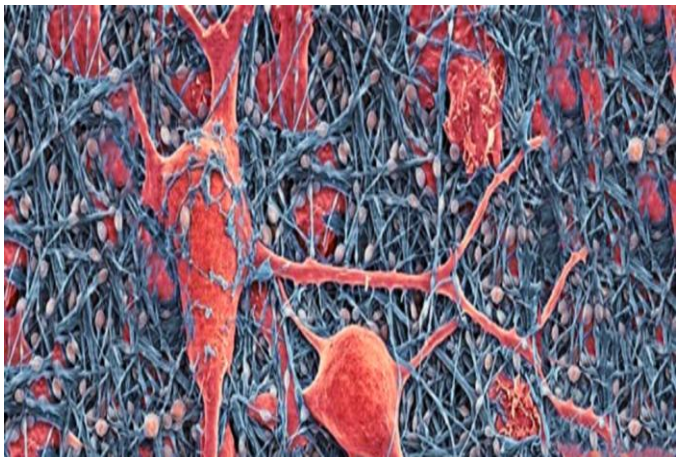

Figure 1.11: Glial cells. Picture adapted from www.retinalmicroscopy.com

Muller cells are also thought to have neural progenitor activity. Retinal tissue autoregulates its blood flow in response to various local and systemic factors through various chemical and cellular interactions (Harris A, 1998). Muller cells regulate the glutamate metabolism, extracellular ionic balance, and neuronal function. Impairment of this autoregulation in diabetes (Sinclair SH, 1982) could lead to derangement of the neural metabolism. Localization of astrocytes is limited to the nerve fibre layer. Their processes wrap around the blood vessels and ganglion cells and play a supportive role in the BRB framework. The interaction of astrocytes is important to induce expression of tight junction proteins and to maintain BRB (Gardner TW, 1997; Abbott NJ, 1992). Together, the macroglial cells integrate the vascular and neuronal activities of the retina. Microglia cells are related to tissue macrophages. They lie quiescent and are activated by homeostatic changes in the retina, which causes them to become phagocytic and help with the immune defence. (Broderick C, 2000; Zeng XX, 2000).

1.2.3 Growth factors:

Various growth factors are involved in regulating the retinal metabolism, including insulin growth factor (IGF-1), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), tissue necrosis factor (TNF), endothelin-1 and hypoxia-induced factor (HIF-1) (Whitmire W, 2011; Ciulla TA, 2003). Excessive additional metabolic stress on the retina, such as occurs in DR, could lead to derangement of the function of these factors and disruption of the BRB system leading to maculopathy and macular oedema.

1.2.4 Retinal circulation:

Retinal circulation differs from cerebral circulation in several important ways. The retinal circulation is sparse, to minimise optical interference. There is large arterioveinous oxygen tension difference. There are no vascular autonomic innervations, and auto regulation is mainly by metabolic feedback. Finally, there are no lymphatics. Fluid balance in the retinal layers completely depends on the equilibrium of hydrostatic and osmotic pressure gradients within the retinal vasculature. The blood-retinal barrier (BRB) selectively permits components into the extracellular fluid; thus, the composition, particularly of the ions, is self-regulated by the retina. In normal retina, there is a capillary-free space around the arteries, but not around the veins, because the partial pressure of oxygen around the arteries is more than that around the veins. Thus, there is no stimulus for capillary growth (Antonetti DA, 1999).

The outer four layers of the retina get their nutrition from choriocapillaries, and the rest of inner layers are supplied by central retinal artery. The outer plexiform layer is partly supplied by choriocapillaries by diffusion, and partly by central retinal artery. The fovea

is an avascular area mainly supplied by choriocapillaries by diffusion. The retinal vessels are end arteries, which do not anastomose with each other. The macula receives its blood supply mainly from the superior and inferior temporal branches of the central retinal artery. In the parafoveal zone the capillary network is well developed and is three-layered. The foveal avascular zone (FAZ) is a capillary free zone of about 500 μm in diameter. Enlargement of FAZ, and the perifoveal intercapillary area, as a measure of capillary density are related to visual acuity and contrast sensitivity (Arend O, 1995; 1997)

1.2.5 Blood-retina barrier (BRB):

The BRB maintains the integrity of the neurosensory retina by compartmentalising and separating the vascular from the neural component. The BRB has two major components. The tight junctions between RPE cells form the outer retinal barrier (Cunha-Vaz JG, 1966). The tight junctional complexes between retinal vascular endothelium and glial cells form the inner BRB (Nishikiori N, 2007). The RPE cells may secrete paracrine vascular cytokines that are important for the survival of adjacent endothelia and maintenance of BRB (Witmer AN, 2003).

1.2.6 Visual function:

Photoreceptors and RPE form the receptor complex, which allows light energy to be transformed into visual stimuli. The outer complex of the photoreceptor faces the long apical microvillus of the RPE and forms a complex junction of interaction. The RPE is responsible for supplying photoreceptors with nutrients such as glucose, retinol, and

fatty acids. Photoreceptors are involved in photoexcitation and phototransduction, through which they convert light energy into neural signals (Strauss O, 2005).

1.3 DMO aetiopathogenesis

Diabetes can be broadly classified into four categories: Type I and II diabetes; Gestational diabetes; Diabetes secondary to genetic defects of beta-cell function or pancreatic diseases; and Diabetes induced by drugs or chemicals (Alberti KG, 1998).

Type I diabetes, which accounts for 5-10% of the diabetic population, is the result of autoimmune destruction of beta cells in the pancreas and lack of insulin production. Type-I diabetes has an immunogenic aetiopathogenesis, and it may be genetically or nongenetically mediated (Atkinson MA, 1994). Environmental factors such as viruses and nutrition may play a role as early as in utero (Leslie RD, 1994), and high birth weight is associated with an increased risk of Type I diabetes (Dahlquist G, 1996).

In contrast to Type I, Type II DM has a multifactorial aetiopathogenesis. It is caused by defects in insulin secretion and insulin sensitivity (resistance). This form of DM accounts for 90-95% of all subjects with diabetes. Changes in nutritional habits, with increased uptake of saturated fats, refined sugars, and alcohol combined with reduced intake of fibres, as well as smoking, family history, ethnicity, and sedentary lifestyle all contribute to Type II diabetes risk (Stumvoll M, 2005).

Gestational diabetes results from glucose intolerance during pregnancy, owing to the excess demand for insulin by the pregnant body.

1.3.1 Abnormalities in insulin secretion:

β-cell dysfunction includes abnormalities in pulsatility and in kinetics of insulin secretion, quantitative and qualitative abnormalities of insulin, β-cell loss and its progression. Both initial peak secretion and later oscillatory release of insulin are affected in Type II DM (Polonsky KS, 1988; O'Rahilly S, 1988). It has been shown that insulin secretary abnormalities start as early as 10 years before the diagnosis of diabetes is made clinically (Whitmire W, 2011; Harris MI, 1992).

1.3.2 Insulin resistance:

Insulin resistance is influenced by both genetic and environmental factors. It is usually associated with visceral obesity, dyslipidemia, hypertension, hyperinsulinemia, impaired fibrinolysis, endothelial dysfunction, vascular inflammation and premature atherosclerosis (Stern M, 1999).

1.3.3 Increased glucagon secretion:

Increased insulin resistance leads to enhanced gluconeogenesis by liver, stimulated by glucagon, and decreased utilisation of glucose by muscle tissue. Glucagon secretion is also stimulated by excessive release of free fatty acids and adipocytokines. Glucagon and vasoactive intestinal peptide (VIP) receptors were found in retinal neuronal amacrine cells in chicks and regulate the proliferation of neural progenitors (Ekman R, 1985; Fischer AJ, 2005).

1.3.4 Pathogenesis of micro vascular retinal complications:

Diabetes mellitus is associated with both macro vascular and micro vascular co-morbidities. Macro vascular complications include cerebro vascular disease, coronary heart disease, and peripheral vascular disease. Micro vascular complications include diabetic retinopathy (DR), diabetic neuropathy, and diabetic nephropathy. Diabetic retinopathy is complicated by diabetic macular oedema (DMO) and proliferative diabetic retinopathy (PDR).

Retinopathy in Type 1 DM is indistinguishable from that in Type 2 DM. The clinical hallmarks of diabetic maculopathy include microaneurysms, haemorrhages, exudates, retinal oedema, which are caused by increased vascular permeability and new vessel proliferation. Fluoroscein angiographic studies have shown that the reduced capillary density (capillary drop) along with an increased foveal avascular zone is correlated with decreased visual ability (Arend O, 1995, 1997) in diabetic retinopathy. There is also capillary dilatation and increased blood flow in early stages of diabetic retina (Grunwald JE, 1994; 1995), which leads to damage of BRB and increased vascular permeability (Cunha-Vaz JG, 1966) and formation of diabetic macular oedema. Other signs of DR progression also follow these changes (Aiello LP, 1998).

Until recently, DMO had been mainly considered a vascular pathology (Curtis TM, 2009; Cunha-Vaz JG, 1975). However, while many studies have emphasized early vascular changes as the cause of DMO, evidence shows that functional and neuronal changes can be identified before development of vascular pathology (Barber AJ, 2003, 2012; Antonetti DA, 2006; van Dijk HW, 2012). These changes are considered to be the direct effect of diabetes on the neural retina, rather than the effects of BRB breakdown.

In the following sections, the micro vascular, neural, and functional changes that lead to macular oedema in a diabetic retina are discussed.

1.3.4.1 Neuronal changes:

Diabetes affects all cell types in the retina (**Table** 1.2) as well as the metabolism of retinal neural tissue. A combination of insulin deficiency, increased apoptosis, glial cell reactivity, microglial activation, and altered glutamate metabolism leads to irreversible neurodegenerative changes, which underlie the functional visual deficits that occur prior to onset of visible retinal changes (Barber AJ, 2003, 2012). Lack of insulin and IGF-1 results in diminished trophic stimuli and activates caspases, resulting in enhanced apoptosis (Whitmire W, 2011). Neuronal death triggers the release of VEGF and other vasoactive cytokines and leads to disruption of the BRB, which is pathognomonic of DMO. Structural modifications of the dendrites occur, including increased length, density, and total number of terminals. These changes exclusively happen in the "ON" ganglion cells (Gastinger MJ, 2008). Loss of retinal ganglion cell bodies is reflected by a reduction in the number of axons in the optic nerve (Scott TM, 1986). Lack of insulin reduces glutamate metabolism in the Muller cells and increases glial fibrillary acidic protein (GFAP) expression (Li Q, 2002). In addition, loss of glial cell efficiency to dispose glutamate causes neurotoxic levels of glutamate to accumulate in the extracellular fluid. Increased glutamate toxicity causes phosphorylation of neurofilaments by inhibiting axonal transport and causing ganglion cell death (Gastinger MJ, 2001). Thus, insulin acts as a survival factor for retinal neurons, and its deficiency directly leads to apoptosis (Barber AJ, 2001; Whitmire W, 2011).

Table 1.2: Retinal cells affected by diabetes

Cell Type	Characteristics
Vascular	Altered tight junctions; endothelial cell and pericyte death
Glial	Altered contacts with vessels; release inflammatory mediators; impaired glutamate metabolism
Microglial	Increased number; release inflammatory mediators
Neurons	Death of ganglion cells, inner nuclear layer; axonal atrophy

Adapted from Gardner TW, 2002

Retinal astrocytes are very sensitive to ischaemia and degeneration. New blood vessels growing at the margin of the perfused and ischaemic retina do not grow in a normal pattern, because of the lack of support from astrocytes. These structural and functional changes are noted in the glia long before changes in the retinal vasculature. Two studies found decreased axonal nerve fibres in the optic nerve and increased glial cell proliferation in experimental rats (Barber AJ, 2003; Scott TM, 1986). Increased glial activity is manifested as the increased expression of GFAP immunoreactivity and content in Muller cells and astrocytes. Glia has a supportive role for neural and vascular elements in the retina (Newman E, 1996); alterations of its function affect the retinal metabolism. VEGF has neuroprotective properties (Jin KL, 2000; Sondell M, 1999), but other, stronger vascular effects have detrimental effects on the diabetic retina.

Current assessment of DMO is based on clinical retinal examination for microvascular changes, which are thought to cause secondary neuronal changes (**Table** 1.3). However, recent evidence (**Table** 1.3) indicates that: 1) neuronal changes predispose to vascular changes, 2) early functional changes are due to the effects of insulin on retinal neuronal tissue, and 3) manifested clinical vascular changes are secondary to neuronal damage.

Table 1.3: Evidence suggesting diabetes induces degeneration of human retinal ganglion cells

Method	Observation	Reference
Histology of autopsy samples	Atrophy of RGC, degeneration of NFL	Wolter JR,1961; Bloodworth JM jr, 1962
Immunohistochemistry of autopsy samples	Apoptosis of RGC, overexpression of Bax and activated caspase-9 and -3	Barber AJ, 1998; Abu-El-Asrar, 2004; Abu El-Asrar, 2007; Oshitari T, 2008
NFL defects detected by red-free photography	Detectable in 20% of diabetics without and 57% with microaneurysms	Chihara E, 1993
NFL "thickness" from scanning laser polarimetry	Decreased in diabetic patients and related to severity of retinopathy	Chihara, 1998; Ozdek, 2002; Takahashi, 2006
NFL "thickness" from scanning laser polarimetry	Decreased in superior retina of diabetic patients	Lopes de Faria JM, 2002

Adapted from Kern TS, Barber AJ. (2008)

Note: RGC: retinal ganglion cells, NFL: nerve fibre layer

1.3.4.2 Circulatory changes:

Altered retinal perfusion and haemodynamics occurs in diabetic macula even before microaneurysm formation and is associated with increased perifoveal inter capillary area as a sign of decreased capillary density. Various studies have found hyper/hypo perfusion to be pathogenic in patients with diabetes (Sakata K, 2006). There is evidence that decrease in retinal perfusion occurs before the onset of diabetic maculopathy followed by gradual increase as the disease progresses. Decrease in perfusion occurs by progressive capillary closure and increased resistance and decreased perfusion (Arend

O, 1991; Greene AS, 1989). This capillary loss and decreased perfusion is associated with decrease in visual function. The effects of hyperperfusion could be explained by the shear stress caused on the vessel walls and induction of humoral factors. (Bresnick GH, 1984; Ashton N, 1963).

The macula has a pulsatile ophthalmic blood flow. Protein kinase C (PKC) and endothelins may play a key role in triggering vasoconstriction and diabetic maculopathy. In contrast, vascular endothelial growth factor (VEGF) released by the ischaemic retina stimulates retinal vasodilatation causing macular oedema and inducing neovascularisation. Although recent clinical treatments (Ford JA, 2013) have been established to reduce or prevent diabetic retinopathy by blocking the pathologic effects of ischaemia induced growth factors and deranged autoregulation, the precise mechanism of various stages of disease progression is not yet clear. VEGF may be the cause for increased choroidal blood flow and macular oedema, and overwhelm the vasoconstrictive effects of PKC and endothelins. There is a contradictory evidence of role of varying blood flow in the onset of diabetic macular oedema (Aiello LP, 2004; Campochiaro PA. 2004; Park JY, 2000; Yokota T, 2003; Aeillo LP, 1994; Chiarelli F, 2000). But the demonstration of altered blood flow across various stages of maculopathy and macular oedema suggest that the growth factors attributed are a part of more complex autoregulatory system along with other unknown factors. The cellular and biochemical mechanisms involving early activation of PKC and endothelin-1 may cause focal vasoconstriction effects leading to focal ischaemia, despite overall increased choroidal circulation in the early stages of maculopathy. The ischaemia indused upregulation of VEGF may be responsible for acute increase in choroidal blood flow

and macular oedema formation in moderate to severe diabetic eye disease. (Savage HI, 2004)

1.3.4.3 Local effects of hypoxia on the rhodopsin cycle:

Michaelson (1954) first identified the causative link between hypoxia and diabetic retinal complicaitons. Chronic retinal hypoxia induces several retinal factors (Cai J, 2002), of which VEGF is the most important (Osborne NN, 2004; Stefansson E, 2006) (**Figure** 1.12). Tissue oxygen tension regulates the production of these growth factors. Improved oxygenation of the inner retina and relief of hypoxia lower retinal VEGF production and hinder further retinopathic changes (Stefansson E, 2006). Hyperoxia reduces VEGF production in the ischaemic retina, thereby decreasing endothelial proliferation (Pournaras CJ, 1997). Hypoxia as the most potent stimulus (Arden GB, 2012), is caused by capillary shut down and also happens in later stages of pathogenesis. Experiments in rodent models have demonstrated early psychophysical changes and electrophysiologic changes before capillary loss could be seen. There is also evidence that hypoxia exists before the onset of capillary shut down (Feit-Leichman RA, 2005; Segawa Y, 1998).

In summary, retinal hypoxia stimulates progression of retinal pathology in two ways: by hypoxia-induced VEGF production and its effects on vascular smooth muscle cells and pericytes; and by autoregulatory dilatation of the capillaries, which directly stimulates growth. Hypoxia has many metabolic sequelae. Oxidative phosphorylation is affected and anaerobic metabolism dominates, leading to altered tissue pH, lactate, and adenosine levels. Hypoxia also induces expression of HIF-1 (Zhu H, 1999), which induces other growth factors, such as VEGF (Caro J, 2001), erythropoietin, basic

fibroblast growth factor, IGF, placental growth factor, and several other factors, to promote new vessel formation (Osborne NN, 2004). Hypoxia can also cause adenosine accumulation, which can lead to vasodilatation and endothelial stretch, thereby stimulating endothelial cell proliferation (Lutty GA, 2003).

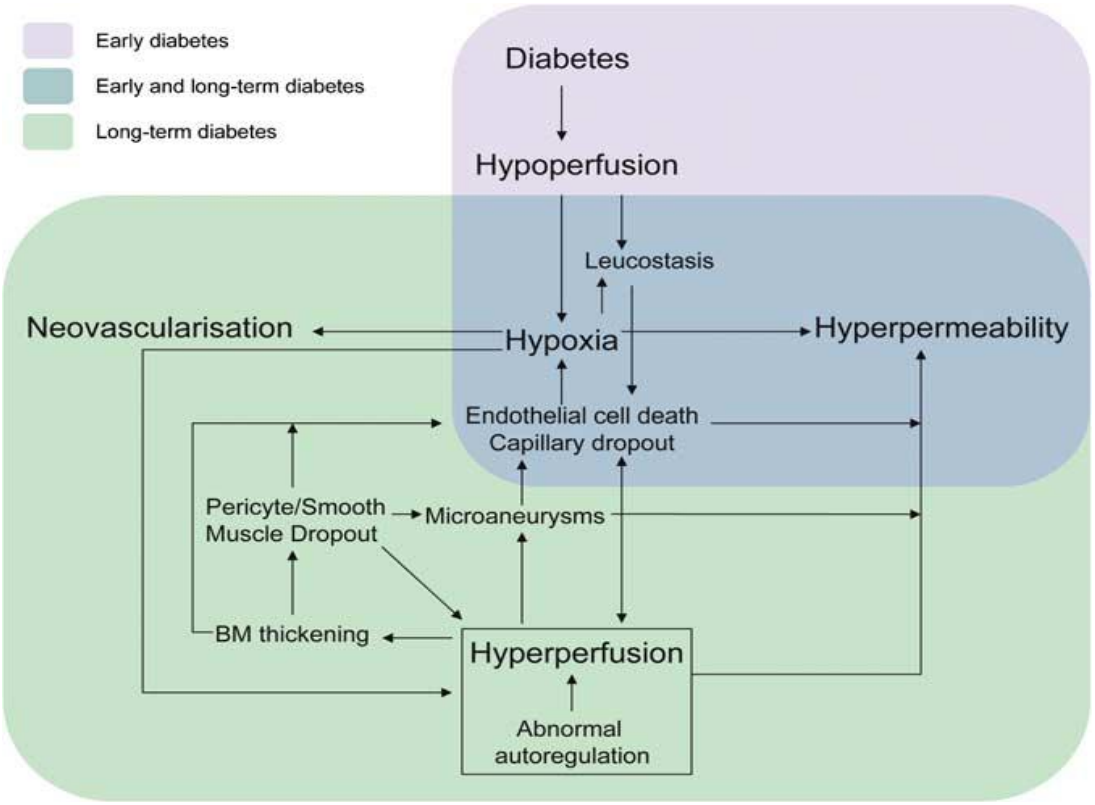

Figure 1.12: Haemodynamic model for pathogenesis of diabetic retinopathy. The purple region shows how early-stage hypoperfusion could lead to progressive hypoxia and increased leucocyte adherence to the retinal capillaries. As diabetes develops, the retinal microvasculature shows hyperperfusion, which leads to BM thickening, loss of arteriolar tone, microaneurysms, and capillary dropout. This process accelerates the hypoxic insult on the retina (adapted from Curtis TM, 2009)

1.3.4.4 Basement membrane thickening:

Increased shear stress resulting from increased blood flow in DR leads to increased endothelial cell gene expression, which in turn causes production of more abnormal basement membrane fibrils and basement membrane thickening. Alterations in protein composition limit communication between cells and contribute to accelerate vascular death. Other underlying mechanisms for increased basement membrane thickening include oxidative stress, polyol pathway flux, activation of protein kinase C (PKC), and accumulation of advanced glycation end products (AGEs) (Stitt AW, 2002a).

1.3.4.5 Loss of pericytes and vascular smooth muscle cells:

In the normal retina, pericytes and endothelial cells exist in a 1:1 ratio. Two-way communication occurs between the endothelial cells and the vascular SMCs (VSMCs)/pericytes to maintain vessel integrity (Armulik A, 2005). Lack of insulin causes less release of IGF-1, which reduces the trophic stimulus to pericytes and leads to degeneration and cell death. Endothelial cells release PDGF for pericyte and VSMC survival (Lindblom P, 2003), whereas pericytes and VSMC in turn express VEGF and angiopoeitin for enhancement of survival and integrity of the endothelium. Basement membrane thickening limits communication between these cells and leads to early cell death. Loss of pericytes could be related to leucocyte adhesion to the vessel wall and accumulation of AGEs; because they express AGE receptors, pericytes may be susceptible to the damaging effects of AGEs (Curtis TM, 2009).

1.3.4.6 Acellular capillary formation:

Pathological levels of shear stress caused by irregularities in blood flow induce endothelial cell death, which can lead to acellular capillary and microaneurysm formation. Leucocyte adhesion to endothelial cells can promote receptor-mediated cell apoptosis. The resultant ischemic environment strongly stimulates VEGF production from various retinal cells, including vascular endothelial cells of the dying capillaries. This VEGF stimulus causes cells to proliferate and form microaneurysms. Capillary drop out initiates from the arterial side, whereas microaneurysm formation develops on the venous side (Poulaki V, 2004).

1.3.4.7 Blood-retinal barrier disruption:

Endothelium, with its junctional proteins (Gardner TW, 1999), comprises the functional part of the BRB. The two most prominent of these proteins are occludins and claudins, which span the plasma membrane and limit fluid flow between the endothelial cells. Other proteins, including the zonula occludens (ZO 1, 2, and 3), organise to form tight junctions by multiple protein interaction domains. Endothelium has very few transport vesicles (Raviola G, 1977).

Diabetes disorganises the functional aspects of the BRB (Antonetti DA, 1998; Barber AJ, 2000). Loss of BRB integrity leads to increased permeability and seepage of fluid into the extracellular spaces. The retinal pumps (RPE cells) can become compromised, which leads to failed maintenance of the retinal extracellular fluid composition, which in turn causes oedema and neuronal damage. The mechanism of BRB breakdown is multifactorial. It is secondary to changes in tight junctions, pericyte loss, endothelial cell loss, retinal vessel leucostasis, upregulation of vescicular transport, increased

permeability of the surface membranes of RPE cells, activation of the AGE receptor, downregulation of glial cell–derived neurotropic factor (GDNF), retinal vessel dilatation, and vitreoretinal traction (Gillies MC, 1997).

1.3.4.8 Leukostasis and microvascular occlusion:

Along with the impaired BRB, microvascular occlusion also plays an important role in the pathogenesis of DMO. Contributing factors include leucostasis (Barouch FC, 2000), microthrombosis (Boeri D, 2001), and extravascular events such as the invasion of Muller cells into the lumen of vessels (Bek T, 1997). It remains unclear whether these occlusions are primary or secondary to changes in neural metabolism.

1.3.4.9 Microaneurysm formation:

Microaneurysms (10-100 mm in size) are thought to result from pericyte loss (Stitt AW, 1995) and vessel wall weakening, which lead to outpouching of the capillaries and breakdown of the inner BRB.

1.3.4.10 Growth factors and proliferative changes:

Progressive retinal hypoxia and ischemia causes release of various angiogenic factors, including cytokines and growth factors from neural, macroglial, and vascular endothelial cells. These factors promote formation of new blood vessels from the venous side and leaking of the capillaries. However, the resulting vessels are fragile and leaky; they bleed and can penetrate through the inner limiting membrane into the vitreous. If leakage is left untreated, the vessels may be replaced by a dense fibrous connective tissue, which adheres firmly to the posterior hyaloid membrane. When this

tissue contracts, it applies traction forces on the vitreal and retinal surfaces and can result in cystoid macular oedema, pre-retinal haemorrhages and traction retinal detachment (Curtis TM, 2009).

1.3.5 Functional changes:

Evidence that diabetic retinal change is an early neurologic pathology is substantiated by various neurophysiological, psychometric, histopathological, and biochemical experiments and can be assessed by electroretinography, visual fields, contrast sensitivity, and colour vision (Sokol S, 1985; Greenstein V, 1990; Falsini B, 1989). Verma A et.al (2009), demonstrated that early spectral domain OCT changes correlate with early functional visual loss, even before clinical signs of DMO develop. Chihara E et.al (1993) noted early nerve fibre layer defects with red-free photography before onset of clinical DMO. Electrophysiological changes in ERG noted in early maculopathy (before onset of microvascular changes) (Parisi V, 2001) are thought to predict worsening retinopathy better than clinically visible retinopathic characteristics (Bresnick GH, 1987).

1.3.6 DMO formation:

Many studies have shown the influence of vasodilatation, elongation, tortuosity, diameter, and transmural pressure on the formation of DMO (Gottfredsdottir MS, 1993; Kristinsson JK, 1997; Kylstra JA, 1986). The net flux of fluid and molecules across the blood vessel wall depends on the difference between the intraluminal hydrostatic pressure which pushes fluid out and plasma colloid osmotic pressure which pulls fluid in (as explained by Starling's law). Increased blood pressure, changes in retinal

metabolism, hypoxia, and other factors can increase the hydrostatic pressure, dilation, tortuosity and can cause blood vessel elongation as explained by LaPlace's law. Lumenal hydrostatic pressure is often increased in diabetic eyes, due in part to coexisting systemic hypertension and in part to the increase in hydrostatic pressure that arises from focal retinal hypoxia. The LaPlace's law states that a vessel will react to increased lumenal hydrostatic pressure by both dilating and becoming more tortuous. As a consequence, tight junctions between endothelial cells may become disrupted, again favouring fluid egress and macular oedema formation. Retinal swelling also initiates the intracellular swelling of Muller cells in the outer plexiform layer (Fine BS, 1981).

1.4 Current concepts postulating pathogenesis of DMO

Various concepts have emerged regarding the pathogenesis of DMO. The two main concepts of interest to this study are whether DMO is a primary vasculopathy causing retinal neural swelling or a diabetic neuropathy causing retinal vascular changes. It is thought that the retinal circulation becomes compromised due to loss of Mural cells by the capillaries, which leads to the formation of ghost vessels. Lack of blood supply to the neural retina leads to necrosis of the neural and glial cells (Chihara E, 1993; Schellini SA, 1995). Dying neural tissue releases various vasoproliferative factors, which stimulate new blood vessel formation. These new blood vessels are incompetent and leak protein and fluid (Klein R, 1992). Impaired autoregulation due to hypoxia is another pathologic mechanism causing increased vascular permeability (Grunwald JE, 1989). The current theories for DMO pathogenesis are classified below.

1.4.1 Theory 1: Diabetic maculopathy and macular oedema is a hyperglycaemia-induced microvascular disease

The first theory claims that hyperglycaemia, through its effect on various local biochemical and metabolic alterations, is the underlying cause for the changes observed in DMO (**Figure** 1.13, **Figure** 1.14). In this theory, the pathogenic pathways include:

- Increased flux through polyol or hexosamine pathways: Such flux is associated with reduced co-factors required in redox reactions with subsequent alterations in the redox state and increased reactive oxygen species causing tissue damage (Tilton RG, 1989).
- Increased accumulation of sorbitol: Sorbitol is dependent on the activity of aldose reductase and its accumulation may impinge on various pathways. Also, sorbitol can destroy pericytes of the retinal capillaries.
- Increased synthesis of diacyl glycerol (DAG) and increased free fatty acids, together with oxidative stress: This combination leads to the overactivation of several isoforms of PKC(Curtis TM,2004; Koya D, 1998), particularly PKC b II.
- Increased production of free radicals: (**Figure** 1.15) This situation leads to oxidative stress (Obrosova IG, 2001; Kowluru RA, 2006), changes in blood rheology and haemodynamics (Tooke JE, 2000; Schmetterer L, 1999), and overactivation of the renin-angiotensin system (Miyata T, 2003). Renin, angiotensin converting enzyme and angiotensin II receptors are widely distributed in the retinal and choroidal vessels and angiotensin has been shown to be angiogenic in animal experiments (Dancer AH, 1989).

- Increased accumulation of AGEs in the vitreous and vitreoretinal interface and activation of receptors for AGEs: These situations lead to increased activation of PKC and hypoperfusion.

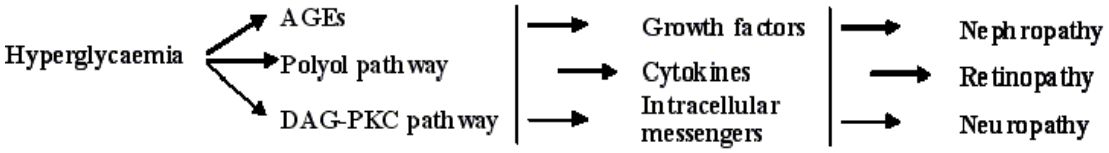

Figure 1.13: Assumed effects of hyperglycaemia **(**Arden GB, 2009**).**

AGE: Advanced Glycation End products, DAG-PKC: Diacyl glycerol-Phospokinase C

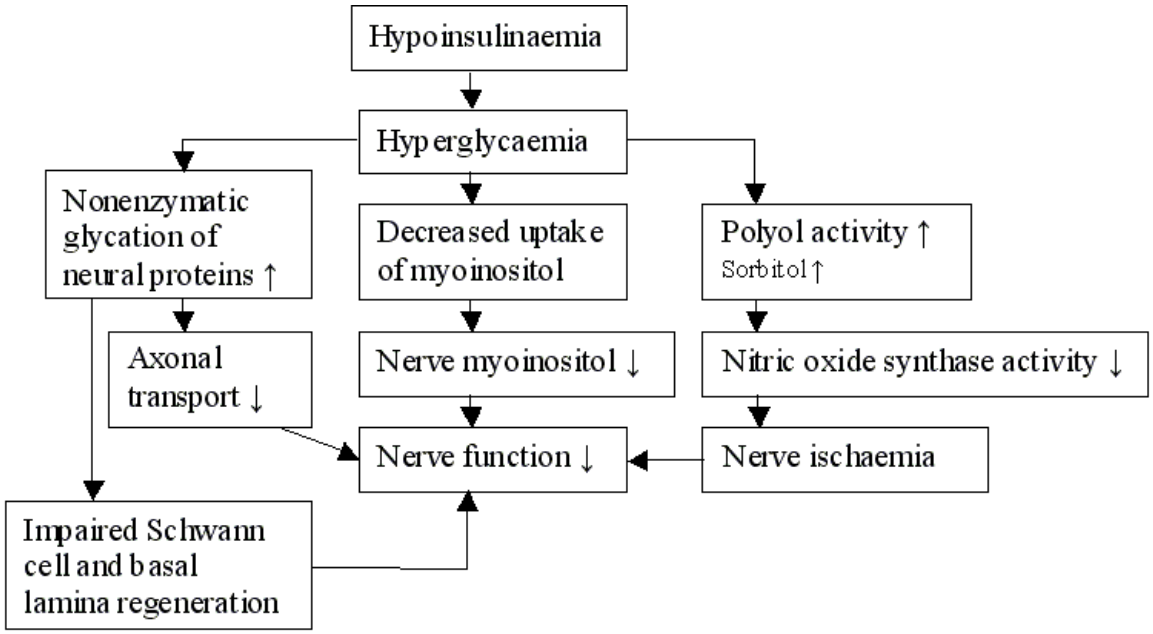

Figure 1.14: Hyperglycaemia-induced neuronal damage (Arden GB, 2009)

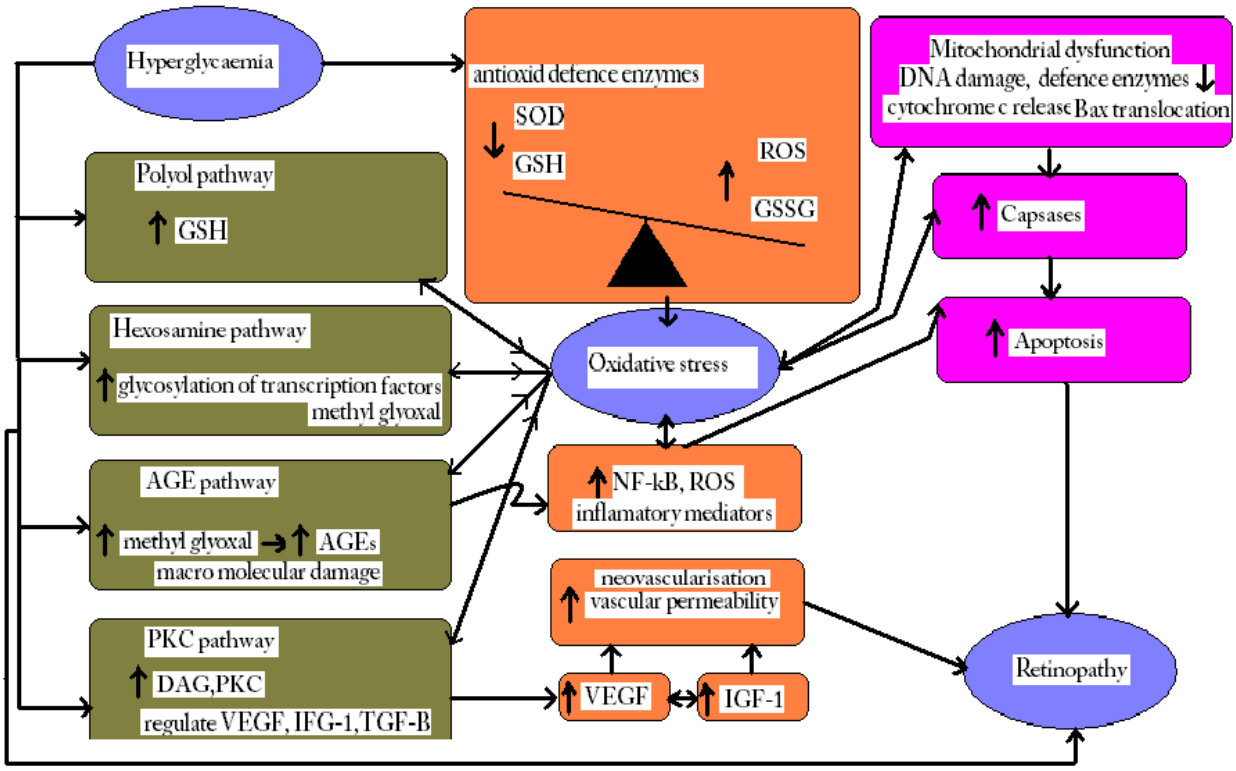

Figure 1.15: Pathways leading to oxidative stress in diabetic retinal changes (Arden GB, 2009). SOD: Superoxide dismutace, GSSG: Oxidized glutathione, ROS: Reactive oxygen species, GSH: Glutathion, AGE: Advanced glycation end products, DAG-PKC: Diacyl glycerol- Phospokinase C, VEGF: Vascular endothelial growth factor, TGF-β: Transforming growth factor beta IFG-1: Insulin like growth factor, NF-Kβ: nuclear factor kappa-light-chain-enhancer of activated B cells, DNA: Deoxyribo nucleic acid

1.4.2 Theory 2: Diabetic macular oedema is a primary retinal neuropathy induced by insulin deficiency leading to vascular changes

Neuronal changes in diabetic retina include apoptosis, glial cell reactivity, microglial activation, altered glutamate metabolism, and altered expression of GFAP in astrocytes and Muller cells. Insulin deficiency is thought to be the primary insult to cause retinal changes and apoptosis of neural cells (Li W, 1997; Barber AJ, 1998; Bloodworth, 1962b; Wolter JR, 1961; Hammes HP, 1995; Barber AJ, 2005). On the basis of this information, others have hypothesised that diabetic macular change is a predominantly

neuronal pathology (Lieth E, 2000). Diabetes induces chronic neurodegenerative changes in the retina, as evidenced by decreasing ganglion cells and thinning of the neural retina on scanning laser polarimetry (Lopes de Faria JM, 2002). Loss of ganglion cells is followed by reduction in the number of axons in the optic nerve (Scott TM, 1986), coupled with a deficit in retrograde transport in the optic nerve (Zhang L, 1998), which together cause functional loss of the retina.

Over 50 years ago, Wolter JR, (1961) and Bloodworth JM (1962a) noted pyknosis in histological sections of the diabetic retina. Apoptosis was also noted in the endothelial cells in later studies (Mizutani M, 1998). Pyknosis is irreversible condensation of chromatin in cells undergoing apoptosis, which is a programmed cell death. Necrosis is a type of cell injury, which leads to premature death of cells in a living tissue. It is caused by factors external to the cell such as infection, toxins or trauma that result in upregulated digestion of the cell components. It could be that increased oxygen consumption in the metabolically highly active retina leads to a hypoxic environment in already compromised retina (Alder VA, 1997), which in turn causes neural cell death and release of various vasoactive factors. Immunohistochemical evidence that apoptosis occurs in the diabetic retina could be derived from the observed enhanced expression of Bax, caspase-9, and caspase-3 (Oshitari T, 2008). Structural remodelling of the dendrites and increases in the total length, density, and number of terminals are seen in large ON-retinal ganglion cells (Qin Y, 2006; Meyer-Rusenberg B, 2007).

1.5 Current and new diagnostics

Various diagnostic tools are used for the assessment of DMO severity. This section, discusses currently utilized and new diagnostics for DMO.

1.5.1 Clinical examination:

Clinical examination for DMO is a subjective and highly variable assessment (Hee et al., 1998) that is no longer considered exclusively when making the decision to treat. Because clinical signs of retinopathy are only seen at the late stages, clinical exam findings represent the terminal stages of retinopathy. Functional losses, such as loss of colour and contrast sensitivities, have already occurred by the time clinical manifestations are seen. For these reasons, clinical examination alone is not useful for early screening and intervention. **Table** 1.4 shows the currently used classification scheme for DR severity by the early-treatment DR study (ETDRS).

Diabetic macular oedema (DMO) is defined as retinal thickening as assessed by dilated examination using slit-lamp bio-microscopy and/or stereo fundus photography. According to the English retinopathy minimum grading classification, maculopathy (M1) is defined as: exudate within 1 disc diameter (DD) of the centre of fovea; circinate or group of exudates within the macula; retinal thickening within 1 DD of the centre of the fovea; and any microaneurysm or haemorrhage within 1 DD of the centre of the fovea only if associated with a best visual acuity (VA) ≤ 6/12. According to ETDRS, clinically significant macular oedema (CSMO) is defined as: retinal thickening within 500 μm of the fovea; hard exudates within the same 500 μm, if associated with retinal thickening; and ≥1 DD of retinal thickening, if any part of the thickened retina is within 1 DD from the fovea.

Table 1.4: Diabetic retinopathy disease severity scale

Proposed Disease Severity Level	Findings Observable on Dilated Ophthalmoscopy
No apparent retinopathy	No abnormalities
Mild nonproliferative DR	Haemorrhages and microaneurysms only
Moderate nonproliferative DR[1]	Extensive microaneurysms, intraretinal haemorrhage, and hard exudates
Severe nonproliferative DR	Any of the following: >20 intraretinal haemorrhages in each of 4 quadrants; definite venous beading in 2 quadrants; prominent intraretinal microvascular abnormalities in 1 quadrant; and no sign of proliferative retinopathy
Proliferative DR	One or more of the following: neovascularization and vitreous/preretinal haemorrhage
Maculopathy	Exudate within 1 disc diameter (DD) of the centre of fovea; circinate or group of exudates within the macula; retinal thickening within 1 DD of the centre of the fovea; and any microaneurysm or haemorrhage within 1 DD of the centre of the fovea only if associated with a best visual acuity (VA) ≤ 6/12
CSMO	Retinal thickening within 500 μm of the fovea; hard exudates within the same 500 μm, if associated with retinal thickening; and ≥1 DD of retinal thickening, if any part of the thickened retina is within 1 DD from the fovea

Adapted from ETDRS Research Group, 1991. [1]Moderate nonproliferative DR was previously termed as “mild preproliferative DR”.

The International Clinical Classification of severity of DMO broadly categorises DMO into two main groups: absent or present. If DMO is present, then it is divided into three groups based on the location of retinal thickening or hard exudates from the centre of the macula.

a) Focal or diffuse macular oedema: Areas of leakage, which may be well circumscribed or diffuse. Focal oedema is often associated with circiante rings of hard exudates, which are leaked out proteins from microaneurysms. Hard exudate is a sign of current or previous macular oedema and often is used as a surrogate marker of DMO on single or 2-field non-stereo photographs. Diffuse oedema represents more extensive break down of blood retinal barrier, with leakage from both microaneurysms and retinal capillaries. Cystic changes appear within the macula, representing coalescence of exudative fluid.
b) Ischaemic maculopathy: the clinical appearance may be relatively normal but the visual acuity has dropped and ischaemia is seen on fluorescein angiography.
c) Clinically significant macular oedema (CSMO): there may be thickening of the retina and hard exudates which, when found within a specific distance of the fovea or when found to be above a certain size, define CSMO.

A severity assessment scale has been published recently for diabetic macular oedema using ETDRS data correlating visual acuities with retinal thickening. Progressive loss of visual acuities were noted with increased severity and duration of DMO, and concluded the likely cause for visual improvement in laser treatment patients is owing to decrease in retinal thickness and duration of diabetic macualar oedema. (Gangnon RE, 2008)

1.5.2 Imaging assessment tools:

Several imaging assessment methods are used in the diagnosis of DMO, including clinical photography, vitreous fluorophotometry (Cunha-Vaz JG, 1979; Raines MF, 1988), fundus fluorescein angiogram (FFA) (Gao LQ, 2008), and optical coherence tomography (OCT) (Verma A, 2009). These methods provide reliable and reproducible objective images that permit quantitative assessment of DMO (Forooghian F, 2008). However, none of them can be used to assess functional loss in the very early stages after diabetes onset, which typically takes the form of a reduction in night vision (Bailey CC, 2001). Such early functional deficits are thought to result from the direct effects of diabetes, with its dysregulation of insulin and IGF, on the neural retina (Lieth E, 2000).

1.5.2.1 Stereo fundus photography:

Stereo fundus imaging is a very sensitive tool for detecting macular oedema that serves as a commonly used screening method in the current management of diabetic maculopathy. However, stereo fundus imaging is associated with several disadvantages. Skilled personnel are required for obtaining and grading the fundus photographs. It is difficult to determine the fundus thickness precisely, and measurements are highly dependent on the subjective stereopsis and picture quality. Given the late onset of clinical signs, the 2-dimensional (2D) nature of the pictures may not be useful in the quantitative evaluation of DMO. This technique also does not provide information on the functional vision.

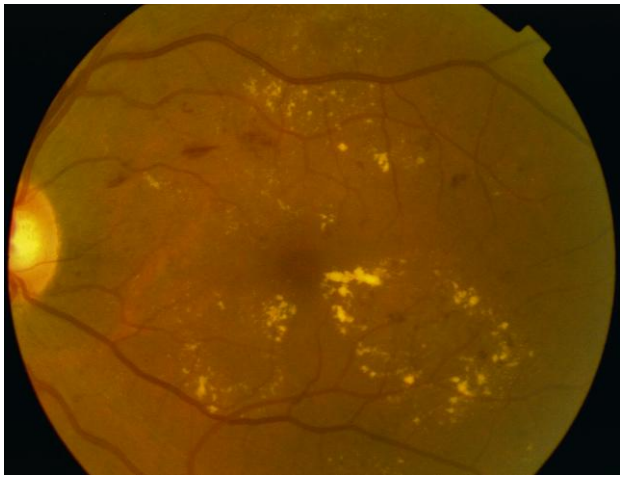

Figure 1.16: Fundus picture of diabetic macular oedema (DMO). A left colour fundus photograph showing exudates, haemorrhages in the macular region.

1.5.2.2 Optical coherence tomography (OCT):

As a more objective assessment than stereo fundus imaging, OCT provides a 3D, cross-sectional orientation of the retinal structures (Shahidi M, 1991)(**Figure** 1.17). Spectral domain OCT, a newer generation high-resolution OCT with high-speed data acquisition and 3D reconstruction of the acquired retinal images, allows improved visualisation of the retinal architecture (Wojtkowski M, 2003; Schmidt-Erfurth U, 2005). Late-onset macular changes can be assessed accurately with OCT; unfortunately, they do not correlate well with clinical changes, because there are high false positive and false negative rates in detecting DMO (Soliman W, 2008). Also, OCT findings do not correlate with functional visual changes, which happen much earlier than detectable macular structural changes. For these reasons, OCT analysis may not be accurate in depicting early functional loss.

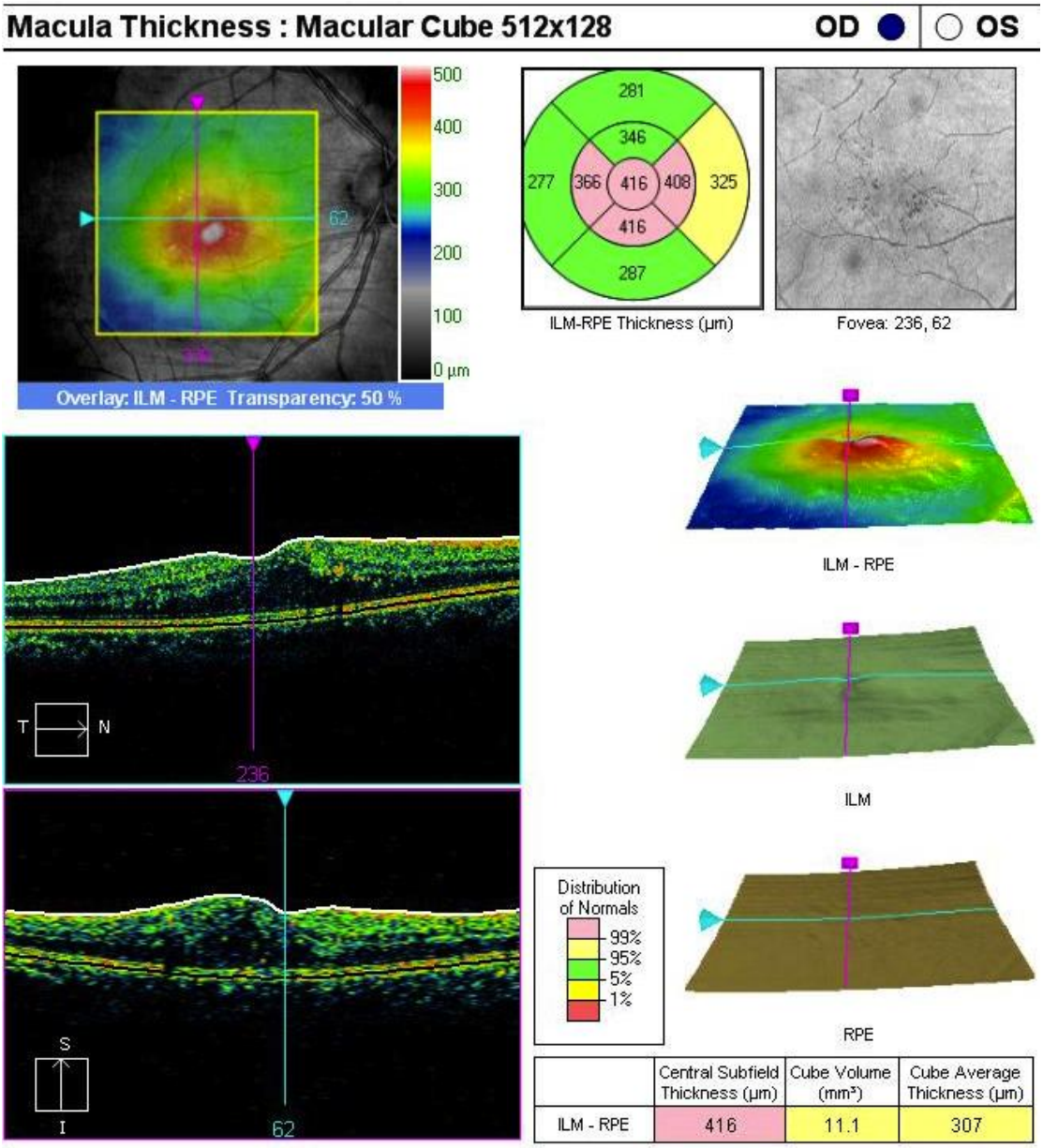

Figure 1.17: OCT picture of DMO. Top left hand corner of the picture shows colour coding of the thickness, top right showing the thickness in microns. The bottom left part of the picture gives an optical cross section of the retina showing retinal cystic changes. The box at the bottom shows CST, macular volume and average thickness.

Scanning with the Cirrus HD-OCT (Cirrus, Carl Zeiss Meditec AG, Jena, Germany) was performed with the 512 × 128 scan pattern, in which a 6 mm × 6 mm macular grid was scanned with 128 horizontal B-scan lines, each consisting of 512 A-scans per line (total of 65,536 sampled points). The macular grid was centred on the intrinsic fixation target during OCT scanning. Decentration of the grid by the technician to centre the grid on the fovea was not allowed. Only patients with scans of signal strength ≥ 6 were included.

The built-in software calculates the average macular thickness (AMT) of all 9 ETDRS-like zones, CST (central subfoveal thickness), and the macular volume. Morphological characteristics of the intraretinal cysts (hyporeflective areas within the retina) were classified as "present" or "absent" at baseline, and improvement is defined as cysts becoming smaller in size, or disappearing altogether; and deterioration defined as appearance of new cyst or larger than baseline. Intrinsic retinal segmentation algorithms were used to define an internal and external retinal layer position from which retinal thickness and volume measurements were derived. In the computational software, the average retinal thickness is the mean thickness in the 9 retinal subfields in a 6-mm-diameter circle centred on the fovea. The central subfield thickness was used for comparison. **Figure** 1.17 shows the OCT scan of foveal region.

1.5.2.3 Fundus fluorescein angiogram:

The FFA method is an objective assessment that provides very good evaluation of the structural integrity of the macula and angiographic evidence of early and late clinical changes. However, this method is time-consuming, requires technical expertise, and cannot be used in all cases because of systemic effects (Soliman W, 2008).

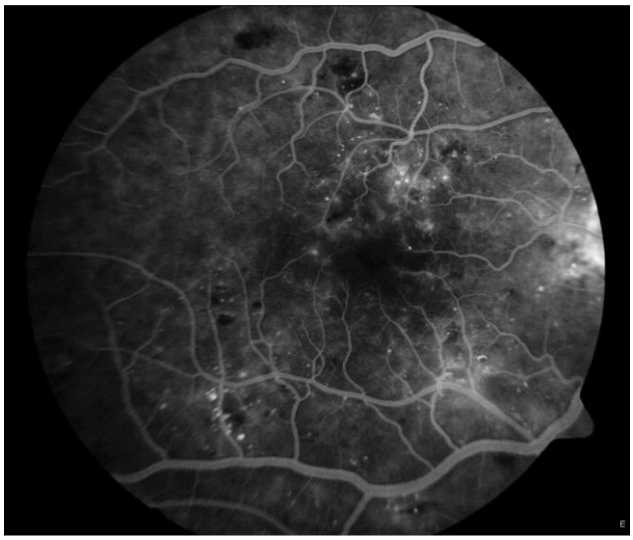

Figure 1.18: Fundus Fluoroscein Angiogram (FFA) of diabetic macular oedema (DMO). Areas of capillary drop outs can be seen at the top corner of the picture and in the middle disruption of foveal avascular zone and leakage from microaneurysms.

1.5.3 Psychophysical tests:

With the rise in Type II diabetes in obese adolescents due to dietary and lifestyle changes, the need for an optimal screening method for sight-threatening DR (STDR) has become imperative (Caprio S, 1999). Early treatment of proliferative DR and diabetic maculopathy improves visual outcome; however, STDR should be detected before visual damage has occurred, because few patients show improvement in vision after laser treatment (ETDRS report 1, 1985)

1.5.3.1 Visual acuity:

Detection of presymptomatic STDR remains difficult. Visual acuity (VA) is a poor predictor of the presence or absence of diabetic maculopathy (Ang GS, 2006); it gives a gross assessment of visual status, but does not correlate well with structural or other functional changes. Visual acuity is only affected in the late stages of DR, when there is

direct foveal involvement in DMO. In contrast with VA, colour contrast sensitivity (CS), achromatic contrast sensitivity, microperimetry (MP), and electrophysiological tests are clinically viable methods to detect functional visual loss before the onset of clinical DR.

The VA measurements all through this thesis were done using ETDRS charts at 4-m distance. These charts are modified versions of the logarithm of the minimum angle of resolution (logMAR) charts designed by Bailey and Lovie. (Lighthouse International, New York, NY). Charts were presented in a light box (Lighthouse International) with a luminance of 150 cd/m^2, as measured with a digital light meter. The settings were inspected regularly by the research standards committee. The authors also performed a random check of VA measurement and luminance.

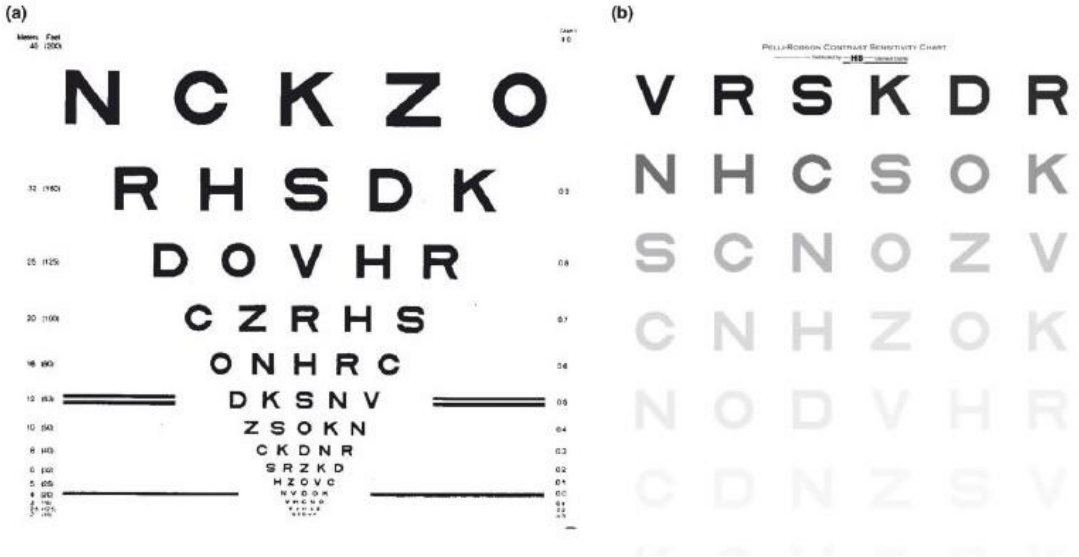

Figure 1.19: ETDRS chart (Image adapted from Colenbrander, 'Measuring Vision and Vision Loss', www.ski.org/Colenbrander/Images/Measuring_Vis_Duane01.pdf. and pelli robson chart (Image provided by Denis Pelli.)

Patients were asked to read and guess each letter on the chart until they made ≥4 mistakes on a single line. The ETDRS VA score was calculated by adding 30 to the number of letters read. If the patients read <4 letters, then adjustments were made for reading the chart from 1 m, according to the protocols. In this case, the final acuity score was obtained by adding the number of letters read correctly at 1 m (up to a maximum of 30 letters) to the number of letters correctly read at 4 m (Patel, 2008). This procedure was repeated at the 3- and 6-month follow-up visits.

1.5.3.2 Chroma test:

Colour-vision testing provides a sensitive, noninvasive method to assess macular damage. Deterioration in colour vision often precedes changes in other clinical measures, such as VA and morphological changes (Hardy KJ, 1992). Several studies have shown a correlation between tritan colour-vision deficiency and DR stage (Bresnick GH, 1985). There is evidence that protan and tritan colour vision is diminished in patients with diabetic maculopathy. However, testing with the FM100 hue and Farnsworth-Lanthony D-15 test are labour-intensive and time-consuming (Bresnick GH, 1985). Colour-vision testing with a computer graphics system is an effective alternative (Wong R, 2008).

Although the mechanism of altered colour vision is unknown, there is evidence that reduced retinal oxygen saturation is associated with impaired colour vision in diabetics (Dean FM, 1997). Error scores in colour vision are directly correlated to the severity of macular oedema (Verriest G, 1982), and selective loss of short wavelength pathway sensitivity is associated with severity of DMO (Greenstein V, 1990; Ueda M, 1992). The chroma test can be used as a quick supplementary test for detecting and monitoring

sight-threatening pathology (Wong R, 2008). Because it measures visual function (rather than features associated with visual loss), the colour vision test could be used for the early identification of subjects who will progress to more severe retinal disease (Ong GL, 2003).

Figure 1.20: Computer set up for Chroma test

1.5.3.3 Contrast testing:

S-cone pathway sensitivity is selectively decreased in the early stages of DR. The deficit in CS appears to be independent of vascular changes in the retina (Sokol S, 1985) and blood glucose levels (Dosso AA, 1998). In particular, blue-yellow contrast and night vision are affected. The fact that hyperoxia improves CS in early DR (Harris A, 1996) indicates that hypoxia could be playing major role in inducing early functional visual changes. Contrast sensitivity examination by Pelli-Robson charts is noninvasive and, in the case of good patient cooperation, can be used to identify functional insufficiency of the retina, which is a sign of initial diabetic changes in the foveolar and perifoveolar regions (Vujosevic S, 2008).

A letter-by-letter scoring method was used to allow better assessment of the repeatability (Elliott, 1990). After corrective refraction (with +0.75 addition),

monocular CS was assessed by a standardized protocol with the Pelli-Robson chart (Clement Clarke Inc., Columbus, OH). Different charts were used for the left and right eyes, at a distance of 1 m and chart luminance of 80–120 cd/m^2. The test was stopped when the patient failed to read ≥2 letters in a triplet (Patel, 2009). The settings were inspected by research standards committee regularly.

1.5.3.4 Electrophysiologic tests:

ERG measures electrical activity within layers of the retina. The ERG results, particularly the amplitude (Juen S, 1990), latency (Sakai H, 1995), and oscillatory potentials (Layton CJ, 2007), are altered in diabetics. These alterations are due to early, reversible physiological changes rather than to permanent structural compromises of the neural retina (Barber AJ, 2003). These changes appear before visible lesions are seen (Fletcher EL, 2007). Oscillatory potentials of ERG reflect the inner retinal neurotransmission (Dong CJ, 2004), mainly from synaptic activity between amacrine neurons and bipolar or retinal ganglion cells (Wachtmeister L, 1998). In diabetics, the oscillatory potentials show prolonged latencies and decreased amplitudes. There is also a progressive delay of visual evoked potentials (Anastasi M, 1985) in the early diabetic retina.

1.5.3.5 Microperimetry:

Microperimetry is a fundus-related perimetry that quantifies functional loss by determining the macular threshold (Okada K, 2006; Mori F, 2002). Although VA is used as the gold standard for visual function evaluation in diabetic patients, it frequently does not correlate with the perception of disability (Midena E, 2007). A better

correlation between early macular pathology and visual function may be achieved by retinal threshold quantification and fixation patterns determination (Carpineto P, 2007). In DR, retinal neurodegeneration may precede photoreceptor loss and reduction of the macular sensitivity may be observed before fixation impairment has occurred (Vujosevic S, 2008).

Microperimetry was performed with the Nidek MP1 microperimeter (MP1 Nidek Technologies, Japan). Because this test requires pupil dilation, it was performed after all of the tests requiring undilated pupils had been completed. At 15 min before microperimetry, the pupils were dilated with a drop of tropicamide 1% and phenylephrine 2.5%. The test was done with one eye patched at a time. In a darkened room, after a briefing trial test was initially performed, the test was performed with a 5-min gap between tests on each eye. All patients had a 30-s fixation test. A 2° red-cross was used as the fixation target. The test stimulus colour was white, Goldmann III size (26 min arc or 0.4°), and duration was 200 ms. Background illumination was set at 1.27 cd/m^2. The intensity of the stimulus ranged 0–20 db, in which "0" represents the brightest luminance (127 cd/m^2).

The perimetric strategy of the MP1 starts at an initially defined threshold level (12 dB) for each stimulus. A 62-loci grid covering central 20° was manually centred on the fovea. If the foveal landmark was not visible on the infrared fundus image, then the foveal centre was located 2 DD temporal and one-third DD inferior to the disc centre, according to the algorithm recommended by Sunness et al, 2007. A 4-2 step strategy was used to reduce the test time and influence of fatigue on the results. The recorded fixation points were classified as "stable" if >75% of the fixation points were inside the 2°-diameter circle; "relatively unstable" if <75% were inside the 2°-diameter circle, but

>75% were inside the 4°-diameter circle; and “unstable” if <75% of the fixation sites were inside the 4°-diameter circle (fujiii, 2003).

Because the mean sensitivity of the perimetry does not provide spatial information, the decision to treat macular disorders is usually based on proximity of the lesion to the centre of fovea. Mean sensitivity of the perimetry was determined as the mean sensitivity of the 62 stimuli. The microperimetry thresholds were also divided based on the 9 ETDRS grid zones. An ETDRS grid was overlaid onto the microperimetry report chart, and thresholds were calculated separately for the central 1-mm zone and remaining 8 zones individually (**Figure** 1.21). Pointwise sensitivities of all of the points in the outer zones were summed to determine the peripheral sensitivity. The 5 points within the central 1-mm zone were summed to get the central zone sensitivity. Zones 2 to 5 were summed to get the parafoveal sensitivities and zones 6 to 9 for the perifoveal sensitivities. The follow-up protocol at 6 months was similar to the first. Automated alignment of the infrared images was ensured either automatically by the software or by manual registration. The central macular sensitivity, defined as the central 16 loci comprising a 4 × 4 grid (5° circle around central of macula) was recorded, because this area corresponded best with the OCT characteristics.

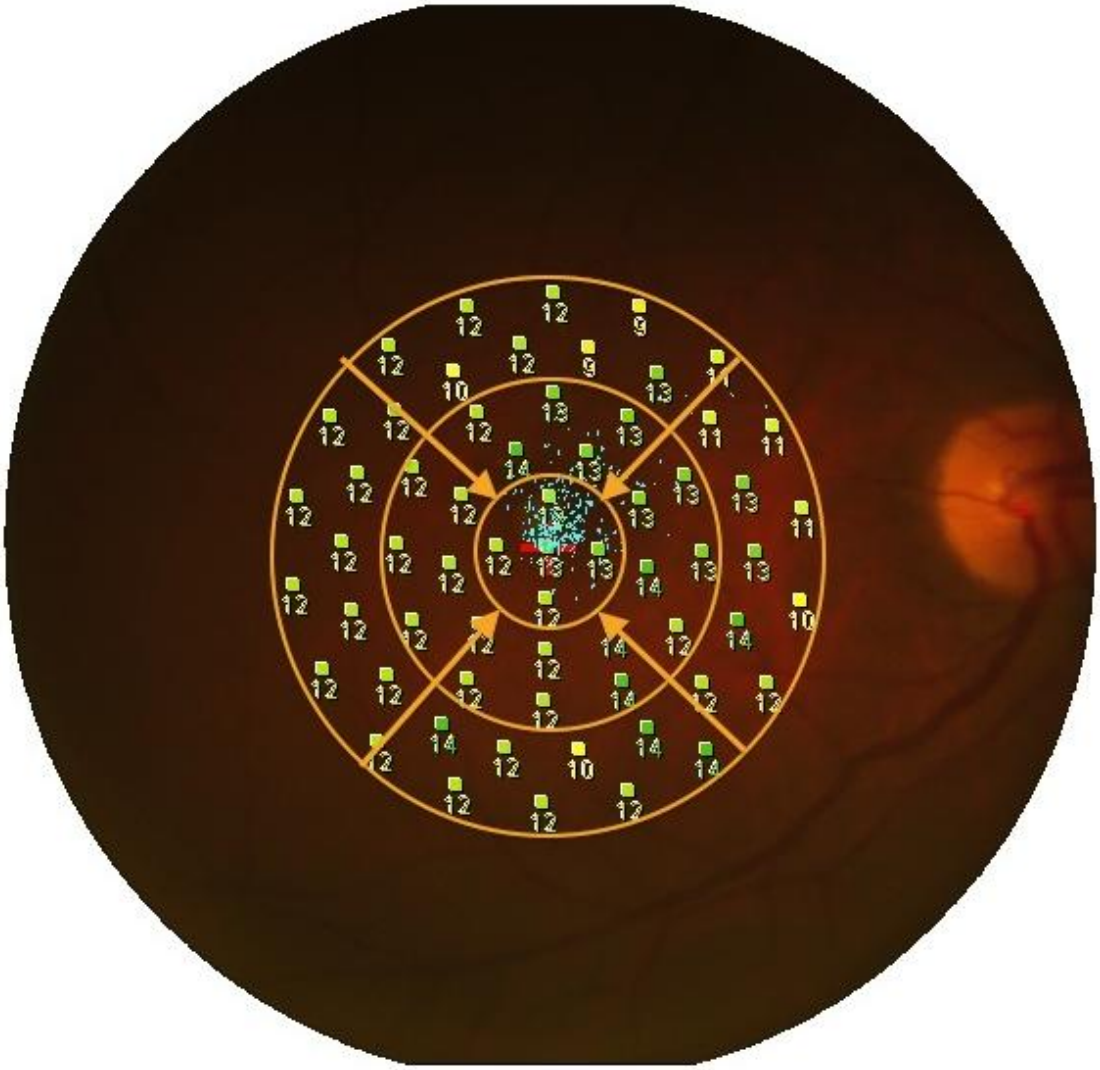

Figure 1.21**:** ETDRS grid overlap on MP1. Inner circle represents central 1 mm of macula, and the outer most circle covers 6mm. The numerals represent point wise sensitivities. Central blue dots represent patient eye tracking.

1.6 Current and new treatments

All of the current treatments for DR are based on addressing visible clinical manifestations. Although laser photocoagulation is the gold standard for DR treatment, it is associated with certain disadvantages. New pharmaceutical modalities are under

investigation, including steroids, anti-VEGF antibodies, PKC inhibitors, VEGF aptamers, aldose reductase inhibitors, and antihistamines. Surgical innovations to treat DR have been evaluated, such as pancreatic transplantation (to increase endogenous insulin) and bariatric surgery (to increase GLP-1). The hypothesis underlying these treatments is that insulin deficiency is the primary insult that causes retinal changes. However, it is difficult to separate insulin deficiency from hyperglycaemia.

Although some of the above treatment modalities have shown promising effects on DR of various stages, their influence on retinal neural tissue and ability to preserve functional aspects of vision have yet to be evaluated. In this section, current and new treatments for DR are discussed.

1.6.1 Laser photocoagulation:

Although various theories have been offered to explain the actual effect of laser photocoagulation on DR, it remains unclear how this treatment modality works. Michaelson (1948) proposed that the ischaemic retina produces a growth factor that promotes neovascularisation. By destroying the ischaemic retina, laser treatment is thought to reduce expression of the neovascularising growth factor and to reverse proliferative changes (Patz A, 1980). However, various post–laser treatment histological studies have suggested that mild to moderate laser destroys the outer retina and pigment epithelium, but does not affect the inner retina (Novack RL, 1990). A second theory proposes that laser-damaged recovering pigment epithelium produces a factor that inhibits neovascularisation (Del Priore LV, 1989, Steffanson E, 2006). A third theory postulates that laser destruction of the photoreceptors reduces oxygen consumption by

the outer retina and increases oxygenation of the inner retina. Choroidal oxygen is believed to diffuse through the laser scars to the inner retina (Stefansson E, 2006).

Regardless of the mechanism, laser increases oxygen tension in the inner retina. The retinal blood supply is largely autoregulated by the chemical composition, mainly oxygen tension (Tomic L, 2005). Increased oxygen tension leads to auto regulatory constriction of the arterioles and reduction of the intravascular hydrostatic pressure, which results in constriction of the capillaries and venules (Wolbarsht ML, 1981). The resultant decreased blood flow (Grunwald JE, 1989) further reduces endothelial stretching, thereby reducing the endothelial growth potential (Stefansson E, 1983).

Before a patient receives laser treatment, irreversible functional visual loss has already occurred. Laser photocoagulation reduces the risk of moderate vision loss by 50%, but only 3% of patients show any improvement; most remain unresponsive (ETDRS Report 1, 1985). Laser photocoagulation can prevent further vision loss in a few, but cannot reverse vision loss and must be repeated (Blankenship GW, 1991). Laser treatment itself is not without adverse effects, including inadvertent foveal burn, central visual field defect, paracentral scotomas, colour vision abnormalities, retinal fibrosis, and spread of laser scars (Lovestam-Adrian M, 2000).

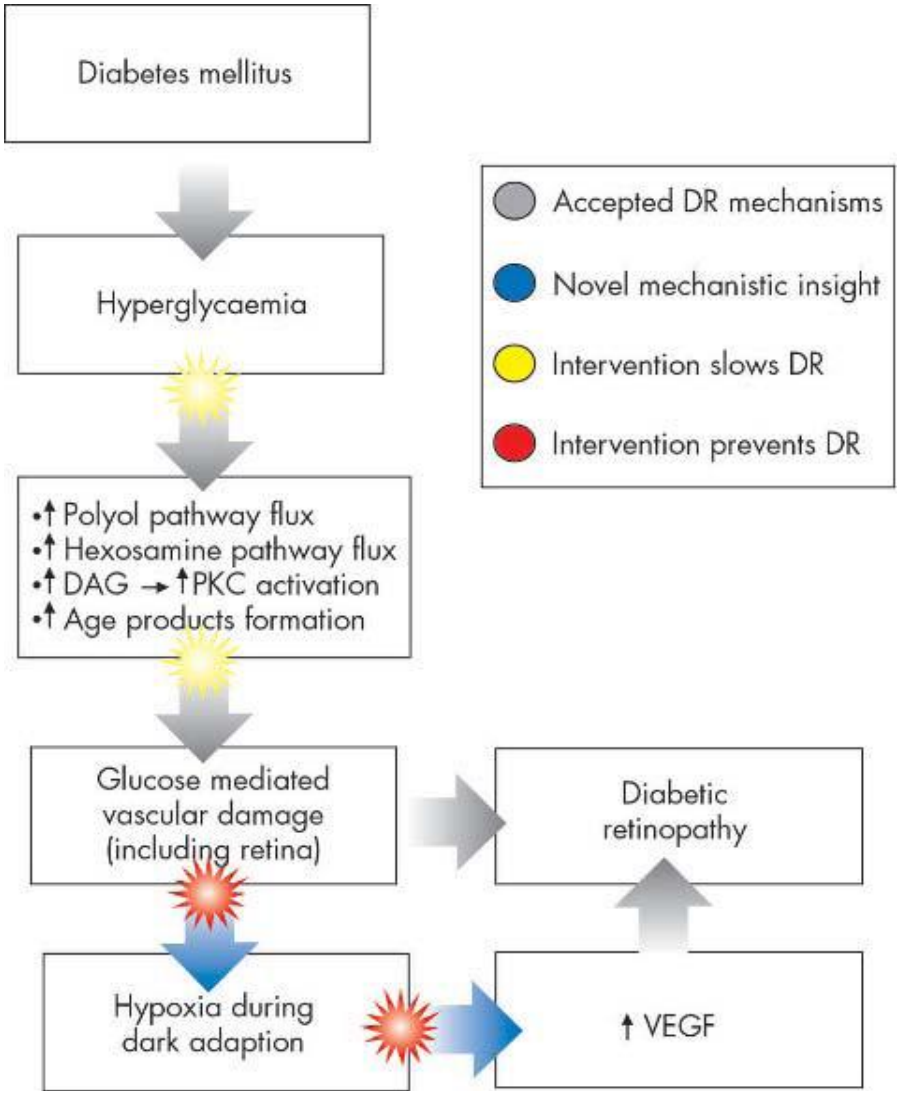

Figure 1.22: Possible therapeutic interventions to prevent DR (Arden GB, 2009)

1.6.2 Addressing dark adaptation induced hypoxia:

It has been proposed that reducing the dark currents of rods can decrease hypoxia and prevent progression of DR (Arden GB, 2005). Rods have the highest metabolic rate of any cell in the body. In the dark, the outer limb membrane leaks, causing an inward dark current of Na^+ and water, which are pumped out in the inner limb. This process is regulated by cGMP and requires substantial energy and a large oxygen supply. Pores in

the rod outer limb membrane close under the influence of light, Ca^{2+} entry is reduced, and the activity of the Na-Ca exchanger diminishes, thereby limiting cGMP utilisation. Results of a Phase I clinical trial indicate that reducing rod cGMP activity by preventing full dark adaptation (i.e., through maintenance of a continuous low level of background light with a light mask at sleep) should decrease peak outer limb retinal oxygen demand, thereby slowing the progression and reversing early changes of DR. (Arden GB, 2010)

1.6.3 Anti-VEGF agents:

Several proinflammatory cytokines (including, in particular, VEGF) are involved in the onset and progression of DMO (Aiello LP, 1997; Poulaki V, 2007). VEGF inhibits endothelial-occludin protein synthesis, causes microvascular leakage, and promotes neovascularisation. Inhibiting VEGF provides an alternate treatment approach to treating DMO. One example therapy is ranibizumab, which is a fully humanized monoclonal antibody fragment (Fab). Various randomised control trials have shown that intravitreal injection of this drug is efficient in reducing DMO and improving visual potential (Nguyen QD, 2010; Massin P, 2010).

1.6.4 Corticosteroids:

There is evidence that inflammation and resulting leukostasis play a vital role in the onset of diabetic macular oedema. Through the release of free radicals, enzymes and cytokines, leukocytes can damage the endothelial cells and there by integrity of the blood retinal barrier. Cytokines released by leukocytes include VEGF, IL-6, TNF- α (Ehrlich R, 2010). ICAM-1 plays a major role in cell-to-cell adhesion and leukostasis. Given the role of inflammation in the pathogenesis of DMO, steroids have been utilized to treat DMO, for its ability to inhibit VEGF production. Corticosteroids mainly act by

interfering and inhibiting expression of proinflammatory genes for TNF α, VEGF and other cytokines (Nauck M, 1998). They also inhibit the phospholipase A2 pathway, and reduce leucocyte chemotaxis.

1.6.5 Glucagon-like peptides:

The retina contains glucagon-like peptide receptors (GLP)-1R, more so in the inner retina, which creates a trophic stimulus on the retinal tissues. This observation has attracted substantial interest, owing to the introduction of synthetic incretins, which are GLP agonists, as part of the treatment armamentarium for diabetes. These GLP agonists can increase insulin sensitivity in the retina, prevent retinal cell death, and maintain normal thickness and so might have an effect on the progression of DR (Zhang Y, 2009).

1.6.6 New antidiabetic drugs:

Table 1.4 shows the latest groups of drugs that are being used in or evaluated for clinical practice (Israili ZH, 2011). Although insulin is the primary target for diabetes treatment, newer antidiabetic agents that only indirectly affect insulin secretion are now available. Some of these agents, such as glitazones, adversely affect DMO. These drugs do not significantly differ from insulin in the control of HbA1C, but have additional effects. Comparison of the effects of these treatment options with insulin on DR may help us to understand the direct effects of insulin on the retina.

Incretin receptor agonists (i.e., exenatide) and enhancers (i.e., sitagliptin) are pharmaceuticals that increase the activity of GLP-1. GLP-1 is an insulinotropic gut peptide that is secreted from the L-cells of the gastrointestinal tract in response to food.

GLP-1 normalizes the blood glucose level, stimulates insulin synthesis, inhibits glucagon secretion, delays gastric emptying, and may promote satiety. It has potent effects on glucose-dependent insulin secretion, insulin gene expression, and pancreatic islet cell formation (Xu G, 2009).

GLP-1R is predominantly expressed in the inner layer of the retina (Zhang Y, 2009), as well as in various other tissues, including pancreatic endocrine cells, intestinal epithelial cells, brain, lung, kidney, heart, mouse skin and primary porcine proximal tubular cells (Baggio LL, 2007). In experimental diabetic rats, the E4 (GLP-1) analogue was found to influence positively retinal electrical responses and to increase thickness of the nerve fibre layer. Thus, GLP-1 analogues might be able to rescue degenerating neurons of DR. Further exploration is needed to determine whether the protective mechanisms come from its glucose-normalising effect or a direct effect on the retina (Zhang Y, 2009).

Table 1.5: Antidiabetic agents and their mode of action (Israili ZH, 2009)

Mode of action	Category of drugs
Peroxisome proliferator–activated receptor gamma agonists; increase peripheral glucose disposal	thiazolidinediones, pioglitazone, and rosiglitazone
Incretin (GLP-) receptor agonists; incretin-mimetics	exenatide and liraglutide
Inhibitors of dipeptidyl-peptidase-4; incretin enhancers	sitagliptin, and vildagliptin
Short-acting, nonsulfonylurea secretagogue; increase pancreatic insulin secretion	meglitinides (repaglinide and nateglinide
Amylin analogue; inhibits glucagon secretion, delays gastric emptying, and acts as a satiety agent	pramlintide

Alpha-glucosidase inhibitors; competitive inhibitors of enzymes needed to digest carbohydrates, reduces carbohydrate metabolism	miglitol and voglibose
Bile acid sequestrant; bind bile acids in the gut and interrupts enterohepatic recirculation of bile acids, effects on hepatic lipoprotein metabolism	colesevelam
Sulfonylureas (SUs); increase pancreatic insulin secretion	glipizide, glyburide
Biguanides; decrease hepatic glucose production	metformin

1.7 Conclusions drawn from literature review

With the global incidence of DM expected to reach 5.4% by 2020, combined with the rise in Type II diabetes among obese adolescents, strategies for managing diabetic co-morbidities such as retinopathy are acutely needed. Macular oedema is the primarily cause of visual impairment in DR, the clinical hallmarks of which include micro-aneurysms, haemorrhages, and exudates resulting from increased vascular permeability and new vessel proliferation. Accumulating evidence indicates that DMO is probably not primarily because of vascular disease, but rather a neurologic disease with far ranging neuro-pathologic, inflammatory, and vasculopathic effects. Early neurologic changes of DR, including functional visual loss, occur much earlier than clinical presentation. Such early effects are thought to result from the direct effects of DM, including hyperglycaemia and/or insulin insufficiency, rather than breakdown of the BRB. Recent successful trials with antibodies to VEGF in diabetic macular oedema indicate the early role of this cytokine in the pathogenesis of DMO. The mechanisms for the raised VEGF levels in diabetic retina are still not clear. Several theories that hypoxia and associated increased reactive oxygen species, collectively referred to as oxidative

stress, could be one of the main causes for early increase in VEGF levels and diabetic macular oedema.

Given the early presentation of functional retinal deficits among patients with diabetic maculopathy, there is a crucial need for early screening methods for sight-threatening DR. Currently used screening methods are based on clinical examination. However, functional vision loss can precede clinical manifestations of DMO by many years. Moreover, use of clinical examination to screen for maculopathy is insufficient. Visual acuity is not a very reliable or functional measure of vision, and no correlation has been found between microvascular changes and VA. Other diagnostic modalities, such as stereo fundus imaging, OCT, and FFA, allow quantitative assessment of DMO; however, they are not appropriate for assessing very early functional loss after onset of maculopathy. Further research is needed to improve diagnostic modalities, so that early intervention can be offered.

In terms of DMO treatments, laser photocoagulation is currently the gold standard. However, laser treatment is not as effective in real-life scenarios as had been predicted from trial setups (DRCRN trials). Although able to reduce the risk of moderate vision loss by 50%, laser photocoagulation improves symptoms in only 3% of patients with DMO (ETDRS Report 1, 1985). The mechanism of action of laser photocoagulation remains unknown, and treatment is associated with important collateral damages, including inadvertent foveal burn, central visual field defect, para central scotomas, colour vision abnormalities, retinal fibrosis, and spread of laser scars (Lovestam-Adrian and Agardh, 2000). Other treatment modalities currently being investigated include the anti-VEGF agent ranibizumab, antidiabetic medications, as well as incretin receptor agonists (exenatide) and enhancers (sitagliptin). Given the emergence of trials assessing

the role of anti-VEGF agents in DMO, there is hope that early diagnosis and intervention are within reach to improve treatment and prevent early functional loss.

1.8 Hypothesis

H1) Laser photocoagulation increases oxygen tension in the inner retina and improve psychophysiologic functions. A long-term laser outcome study is conducted to test this hypothesis (section 2.1)

H2) Hypoxia contributes to the pathogenesis and aggravation of DR and DMO, and photoreceptors utilize oxygen maximally during dark adaptation. So thereotically decreasing oxygen consumption of the photoreceptors by reducing dark adaptation should have a positive impact on diabetic maculopathy. This hypothesis is tested by light adapting the rods and assessing the impact on diabetic maculopathy (Section 2.2)

H3) Neuronal changes precede vascular changes in DMO. This is tested by correlating functional and anatomical changes as described in section 3.

1.9 Aims, objectives and Methodology

1.9.1 Aims:

The aims of the projects contributing to this thesis were to:

1) Assess the efficacy of laser treatments (standard of care) for diabetic maculopathy;
2) Investigate new treatment strategies that reduce the hypoxic insult in diabetic eyes;

3) Assess new diagnostic methods in evaluating the progression of diabetic macular oedema.

1.9.2 Objectives and proposed methodology:

1) To test H1, we assessed the long-term (5-year) outcome of laser photocoagulation. This was achieved by a retrospective analysis of the outcome of laser treatment in over 100 patients.
2) To test H2, we conducted a trial to enhance oxygen availability through inhibition of rod dark adaptation, and assessed whether this approach has any influence on the progression of diabetic maculopathy. This was achieved by a prospective clinical trial performed on 40 patients with early diabetic maculopathy with the use of light-masks to decrease dark adaptation.
3) To test H3, we assessed whether Contrast sensitivity (CS), Colour vision (Chroma test) and Microperimetry (MP) could detect diabetic macular oedema before clinical changes happen and if these tests could be used as screening tools for assessing diabetic macular oedema.

1.9.3 Organization of the thesis:

The overall aim of this thesis is to investigate novel concepts in the diagnosis and treatment of DMO. The thesis is organized as follows. In Chapter 2, I discuss treatment methods for DMO. Section 2.1 presents the long-term (5-year) results of a laser photocoagulation study for DMO and tests hypothesis **H1**. In Section 2.2, I propose a rod dark adaptation approach for early intervention of diabetic maculopathy. A clinical

trial (phase II) was performed to see if inhibiting rod dark adaptation activity by light exposure would influence diabetic maculopathy, thus testing hypothesis **H2**.

In Chapter 3, I discuss the diagnosis of early diabetic maculopathy. I assess use of MP1, colour vision testing, and chroma test (section 3) for ascertaining early functional vision loss before major clinical changes are noted as a test for **H3**. Also in this chapter I monitor the structural and functional changes in eyes with mild maculopathy (with cysts) over 6 months. Chapter 4 discusses the implications of the collective findings, and Chapter 5 concludes the work.

2: TREATING DIABETIC MAULAR OEDEMA

2.1 Five-year visual outcome following laser photocoagulation of diabetic macular oedema

2.1.1 Introduction:

Diabetic maculopathy continues to be a leading cause of new onset vision loss worldwide among working age populations (Aiello LP, 2010). The ETDRS

demonstrated that focal or grid laser photocoagulation reduced the risk of moderate visual loss in patients with CSMO by approximately 50% (from 24% to 12%) at 3 years (section 1.7.1), although VA improvement was observed in <3% of cases, based on 15-letter gain at 3 years (ETDRS report 9, 1991). Despite the unsatisfactory outcomes, this treatment remains the gold standard of treatment for CSMO. Indeed, recent clinical trials conducted by the Diabetic Retinopathy Clinical Research Network (DRCRN.net) indicate that the outcomes associated with macular laser treatment have improved significantly (Bressler NM, 2009; DRCRN, 2008). Advances in laser technology and optimisation of glycaemia and blood pressure (BP) control have been attributed to these beneficial outcomes (Browning DJ, 2008). Similarly, contemporary prevalence studies also suggest that the prevalence of DR and its complications is decreasing when compared to the Wisconsin Epidemiologic Study of Diabetic Retinopathy published in 1984 (Klein R, 2008). This decline in DR prevalence is also thought to be due to the enhanced control of systemic factors (Brown JB, 2003; Kempen JH, 2004; Wong TY, 2006).

For over two decades, lessons from the UK Prospective Diabetes Study (UKPDS VIII, 1991) and the Diabetes Control and Complications Trial (DCCT, 1986) studies have governed our clinical practice with regard to the management of DMO. Strict glycaemic and blood pressure control remain the most effective interventions to date. Given that laser increases oxygen tension in the inner retina (Stefansson, 2006), the aim of the study was to test the hypothesis that VA may continue to improve in eyes with laser-treated maculopathy (H1). Given that contemporary clinical trials and prevalence data suggest an improvement in visual outcomes and better control of risk factors, we conducted a retrospective study to assess the 5-year visual outcome associated with

macular laser photocoagulation (2003-2009) in a clinic-based setting in a multi ethnic inner city population. We also determined the effect of systemic factors on visual outcomes to evaluate whether similar outcomes are obtained in a setting where patients have not been standardized, as they would have been in a clinical trial, representing a real-life clinical situation.

2.1.2 Methods:

The Chair of the Institutional Ethics, Research and Development Committee approved the protocol for this study (Appendix J: Kings Research and Development Committee approval). The project was also registered in the Clinical Effectiveness Department of the institution (Kings college Hospital NHS Trust). The study adhered to the tenets of the Declaration of Helsinki. All the laser procedures in the study were carried out either by the trainees at registrar grade, under supervision, or by the consultant ophthalmologists.

2.1.2.1 Study population:

This study was carried out at King's College Hospital, London, where an established diabetic retinopathy-screening programme caters to a 700,000 multiracial community with high levels of social and material deprivation. One-third of the total study population was drawn from black and ethnic minority groups. Individuals were graded as having DMO based on post-mydriatic 2-field colour fundus photographs. Screen-positive patients were referred to retinal clinics, where a clinical examination and additional investigations (e.g: FFA) were performed before laser photocoagulation. OCT was not available at baseline examinations.

2.1.2.2 Study design:

Consecutive patients with Type 2 diabetes and DMO who required their first macular laser photocoagulation in 2003-2004 were identified from the laser register. In bilateral cases, the first eye treated in each patient was included in the study. In cases where both eyes were treated during the same session, the eye with the poorer baseline VA was included. Patients who did not complete the 5-year follow-up were excluded from the study and the reasons for being lost to follow-up were recorded.

2.1.2.3 Laser photocoagulation:

The focal/grid photocoagulation protocols used in the department mirror the DRCRN.net protocols (modified from the original ETDRS protocol) (Aiello LP, 2010). In brief, treatment was performed with a 532-nm green laser light Iridex Oculite GLx (Iridex Corp, California, USA) with a spot size of 75–125 μm and exposure time of 100 ms to obtain a light grey-white (just visible) burn and applied in a focal or grid pattern to cover the area of oedema.

Patients were reviewed every 4–6 months, unless they failed to attend an appointment. Laser treatment was repeated if clinical, angiographic, and, more recently, OCT evidence indicated persistence of macular thickening. No distinction was made between focal or grid lasers in this study, because in clinical practice, many patients tend to have both on long-term follow-ups.

2.1.2.4 Visual acuity:

Initially, VA was recorded with Snellen VA charts, followed by the ETDRS charts at 2 m. All VA recordings were converted to ETDRS scores for this study. The mean annual visual outcome was defined as the average of all VA measurements recorded per year.

2.1.2.5 Co-morbidity:

All annual clinical data regarding ocular and medical history, including laboratory values, were obtained retrospectively from the electronic patient record, clinical files, and laboratory records. Data collected that was related to systemic factors included age at first laser treatment, gender, ethnicity, length of duration of diabetes at baseline, date of initiating insulin therapy, average annual HbA1C levels, mean annual systolic and diastolic blood pressure, number of anti-hypertensive medications at baseline and annually, average annual BMI, history of being on statins, history of cardiovascular co-morbidity, peripheral neuropathy, and foot ulcers. Data collected that was related to ocular features included mean annual visual outcome, grade of DR, date of cataract surgery (if done), number of macular laser treatments in 5 years, date of initiating pan-retinal photocoagulation (if required), history of any other surgical procedures including date, other ocular co-morbidity, number of retinal clinic appointments in 5 years, and the number of appointments the subject failed to attend in 5 years.

2.1.2.6 Statistical analysis:

The primary outcome measures in our patients (KCH cohort) included the mean final change in visual outcome and the annual outcomes up to 5 years; the 3-year outcomes were compared to the outcomes of the laser arm in the DRCRN randomised controlled study that compared intravitreal triamcinolone IVTA with laser photocoagulation for

DMO (DRCRN, 2009). Covariates for the analysis included baseline age, gender, ethnicity, BMI, baseline VA, baseline HbA1C and mean baseline diastolic and systolic BP. The last observation carried forward method was used to assign 45 missing values over the 5-year study period. Data were expressed as percentages, mean values (with standard deviations) or median values. In the univariate analyses, we compared each of these variables using appropriate statistical methods. After the univariate analysis, a multiple regression model of patient characteristics and outcomes was performed to identify the clinical variables associated with gain and loss of vision. "Gain" is considered to be improvement of 10 or more letters, whilst "Loss" of vision is losing 10 or more letters (Aiello LP, 2010). To correct for multiple comparisons, results were only included in the multivariate analyses when the corresponding ***P*** values were <0.01(Aiello LP, 2010). For univariate analysis patients were stratified by visual acuity letter score as >74, 73-69, 68-59, 58-49, 48-39, 38-24, <23 and categorised into one of 3 age groups: 18 to <60, 60 to <70, 70 and older. This strata was chosen to divide the cohort into equivalent size subgroups as done in DRCRN study (Michael S I, 2008). Multiple regression model with backward selection process was used to analyse variables that were significant (P<0.05) in univariate analysis.

2.1.3 Comparison to DRCRN trial outcome:

Baseline characteristics of the study cohort (KCH) are summarised and compared to the DRCRN study population in **Table** 2.1. The mean age of the patients at study baseline was 68.8 years (range 38–91yr, 95% CI 66.6 to 70.9, SD 10.78), with 47 (31%) female and 53 (69%) male patients (**Figure** 2.1). A total of 201 clinical notes were examined to

identify patients who met the criteria for enrolment; causes for exclusion included: lack of adequate follow-up (n = 54), lost to follow-up (n = 32), and mortality (n = 15).

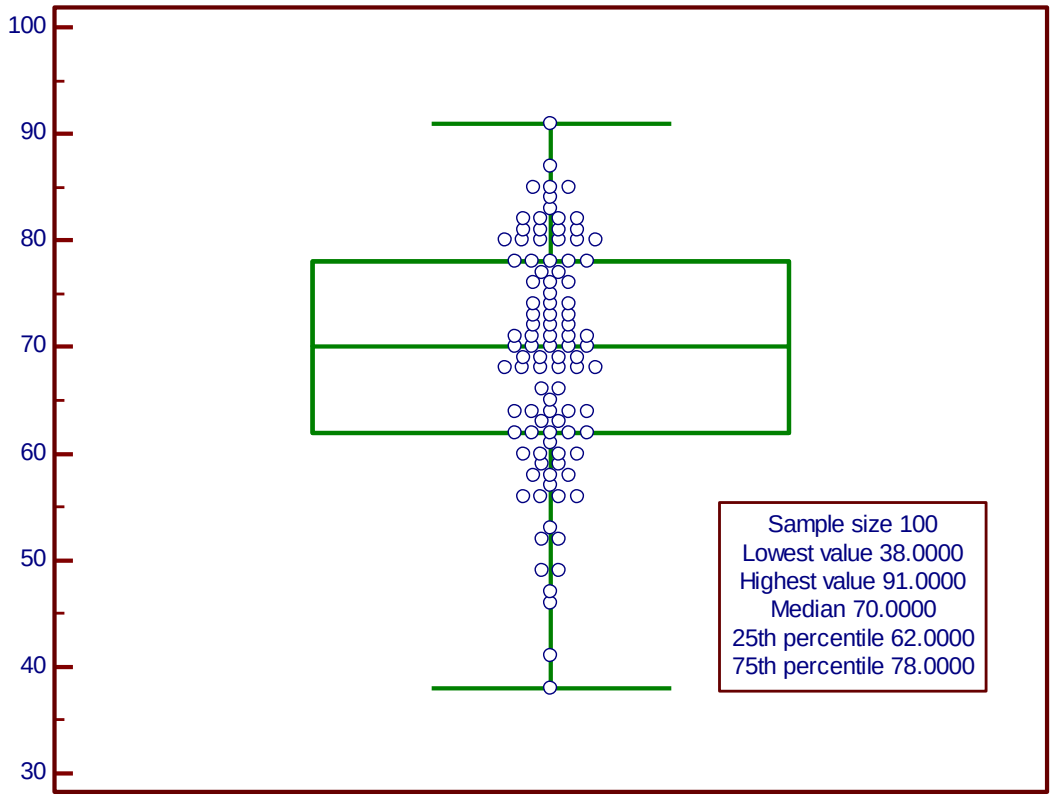

Figure 2.1: Box and Whisker plot of KCH cohort age group.

Table 2.1. Baseline characteristics of the KCH cohort-DRCRN laser group

	DRCRN laser arm	KCH cohort	P(one sample t test, Comparison of proportions)
Number of patients	115	100	
Median age(25^{th},75^{th} percentile)	63(57,69)	60(52,66)	0.0009

Median duration of diabetes at baseline in years (25^{th},75^{th} percentile)	15(10,22)	12(10,17)	=0.07
Mean HbA1C % ± SD	7.9 ± 1.8	9.5 ± 1.9	<0.0001
Prior macular laser at baseline	60%	None	<0.0001
Prior PRPC	16%	3%	=0.0013
Combination PRPC with focal		6%	
Median baseline VA(25^{th},75^{th} percentile)	62(53,67)	70(60,75)	=0.0008
Ethnicity at baseline			
White	74%	38%	<0.0001
Black	9%	47%	<0.0001
Asian	2%	13%	<0.0001
Others	15%	2%	=0.0009
Sex (M: F%)	51:49	54:46	=0.68
Type of diabetes: Type I	4%	0%	=0.08
Type II	96%	100%	=0.08
Phakic at baseline	79%	91%	=0.01
Retinopathy status at baseline			
Mild	58%	85%	<0.0001
Mod	14%	6%	=0.048
Severe	28%	6%	<0.0001
PDR	16%	3%	=0.0013
Hypertensive at baseline	81%	86%	=0.31
CRF	None	11.5%	<0.0001
Cataract surgery	21%	8%	=0.0048

HbA1C: glycosylated haemoglobin; VA: visual acuity; PDR: proliferative diabetic retinopathy.

2.1.4 Results:

2.1.4.1 Visual outcomes:

The mean change in VA at 3 years was -4.15 ETDRS letters in the KCH cohort relative to a gain of 5 ETDRS letters in the DRCRN study. In the first year, the percentages of

gainers were similar in both groups (73% in the KCH cohort versus 74% in the DRCRN laser group). However, by the 3rd year, only 50% of the KCH group patients were gainers compared to 83% in the DRCRN laser group. The proportion of gainers in the KCH cohort was relatively similar from the 3rd to 5th years after the first laser treatment (47–50%; **Table** 2.2). Only one out of 10 KCH cohort members gained ≥15 ETDRS letters at year 1, and this result was maintained to year 5. However, in the DRCRN laser group, the number of patients that gained ≥15 ETDRS letters nearly doubled from 14% in the first year to 26% in the 3rd year. However, the results with the KCH cohort are superior to those of the ETDRS study, in which only 3% gained ≥ 15 ETDRS letters. When we consider the proportion of patients with moderate visual loss at 3 years (loss of ≥ 15 ETDRS letters), the outcomes with the KCH cohort are inferior (27%) to those of the DRCRN laser group (8%). Taken together, the results of these comparisons show that the visual outcomes of the KCH cohort are inferior to the visual outcomes of the laser group in the contemporary DRCRN study (**Figure** 2.2).

2.1.4.2 Mean number of laser treatments:

The mean number of laser treatments over the 5-year study period for the KCH cohort was 2.74 ± 1.6. **Table** 2.3 shows the number of laser treatments for the KCH cohort compared to the DRCRN laser group. The mean number of laser treatments performed was less for the KCH cohort, and more patients in the KCH cohort had only one laser session compared to the DRCRN laser group, despite the fact that 60% of the DRCRN group had prior laser treatment and 13% of the DRCRN group had additional treatments other than laser (e.g., IVTA and bevacizumab). All of the patients in the KCH cohort were treatment-naïve, and none of the KCH cohort patients received any additional

intravitreal treatments. Notably, the proportion of patients having ≥4 laser sessions in the KCH group was less than that in the DRCRN group.

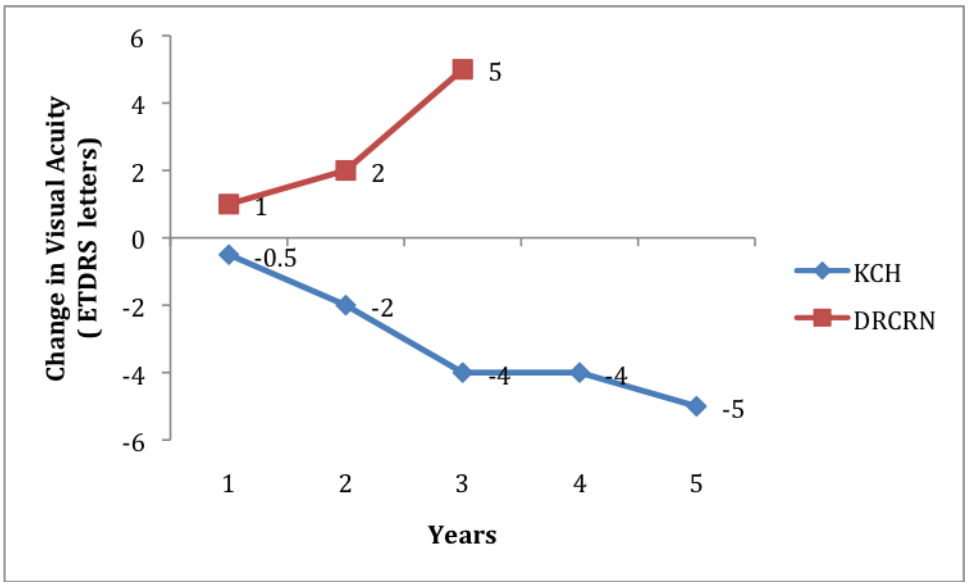

Figure 2.2. Comparison of annual changes in VA between KCH cohort and DRCRN laser group

Table 2.2: Annual mean visual outcomes of the KCH cohort compared to the DRCRN laser group outcomes (DRCRN, 2009)

	KCH 1st year	DRCRN 1st year	KCH 2nd year	DRCRN 2nd year	KCH 3rd year	DRCRN 3rd year	KCH 4th year	KCH 5th year
Changes in VA:								
Mean ± SD	-0.48 ± 11.74	1 ± 16	-2.08 ± 14.62	2 ± 17	-4.15 ± 15.2	5 ± 17	-4.03 ± 15.34	-5.23 ± 17.2
Median (95% CI)	0 (-2.7, 1.8)	3 (-5, 10)	0 (-4.9, 0.79)	5 (-5, 12)	-4 (-7, -1)	8 (-2, 15)	-5 (-7, -1)	-5 (-8.6, -1.8)
≥15 letter gain	10%	14%	10%	20%	9%	26%	13%	12%
10-14 letter gain	11%	14%	6%	14%	5%	18%	2%	4%
5-9 letter gain	10%	17%	18%	17%	14%	18%	12%	9%
no change ±4 letters	42%	29%	27%	22%	22%	21%	20%	22%
5-9 letter loss	6%	9%	8%	9%	12%	4%	17%	16%
10-14 letter loss	7%	3%	10%	6%	11%	4%	8%	10%
>15 letter loss	14%	14%	21%	13%	27%	8%	28%	27%

Table 2.3: Number of laser treatments in KCH cohort compared to DRCRN laser group

Number of laser treatments	DRCRN laser group (3rd year)	KCH cohort (3rd year)	KCH cohort (5th year)
1 session	19	32	23
2 sessions	24	28	32
3 sessions	25	20	21
4 sessions	18	5	8
5 sessions	10	7	8
6 sessions or more	4	8	8
Mean laser sessions	2.9 ± 1.4	2.54 ± 2.0	2.74 ± 1.6

2.1.4.3 Influence of systemic factors on visual outcome at 5 years

Table 2.4 shows the mean annual changes in HbA1C and systolic and diastolic blood pressure in the KCH cohort over 5 years in the current era of improved glycaemia and blood pressure control relative to the DRCRN cohort. Although the mean HbA1C and blood pressure values in the KCH cohort improved slowly over the 5-year study period, the overall control of risk factors for the KCH cohort was inferior to the baseline data for the DRCRN laser group. There were no differences in demographic and systemic parameters between various ethnic groups in the KCH cohort excepting significantly lower diastolic blood pressure in Asian cohort (**Table** 2.5).

Univariate analyses of the known risk factors are shown in **Table** 2.6. For this analysis the visual outcome is categorised "Gainers" who were defined as those gaining 10 or more ETDRS letters; whilst "Losers" were those who lost 10 or more letters. Insulin users, base line BMI, baseline VA, number of laser treatments (a surrogate marker of

DMO severity), number of anti hypertensives and more failed appointments were significantly correlating with change in visual outcome. However, the multivariate model showed that better visual acuity at baseline, worsening of HbA1c, baseline lower diastolic blood pressure and further reduction of diastolic blood pressure were the only poor prognostic indicators (**Table** 2.7).

Table 2.4: Changes in HbA1C and BP in KCH cohort over the 5-year study period.

	Baseline	Year 1	Year 2	Year 3	Year 4	Year 5
HbA1C	9.25±1.99	9.17±2.09	9.4±2.06	8.82±1.87	8.85±1.82	8.7±1.81
	(5.7–15.4)	(5.6–18.6)	(5.8–15.6)	(4.4–13.5)	(5.5–16.8)	(6.2–16.8)
Systolic	143±23.37	142±21.31	144±22.36	142±19.70	140±21.76	141±21.57
BP	(93–234)	(94–195)	(82–200)	(95–190)	(92–200)	(84–204)
Diastolic	80 ± 11.56	79 ± 11.88	78 ± 12.15	77 ± 10.7	75 ± 11.51	77 ± 11.59
BP	(50–122)	(52–110)	(43–105)	(46–105)	(50–108)	(50–108)

SD: standard deviation; HbA1C: glycosylated haemoglobin. Data are shown as mean ± SD (range)

Table 2.5: Differences in demographic and systemic parameters between various ethnic groups in the KCH cohort.

	Caucasians	**Blacks**	**Other ethnic groups**	**P(one way ANOVA)**
No: at baseline (N)	37	47	16	
Age at first laser	59.5	59	60.25	=0.9
Duration of DM in years Mean (range) Median	 14 (2–50) 12	 13 (1–40) 12	 14 (1–34) 13	
Time gap between macular laser and PRP in months	35	30	26	=0.9
No: macular lasers	2.5	3	2.4	=0.7
No: PRP lasers	2.8	2	1.5	=0.7
No: clinic appointments	20	18	19	=0.4
No: failed clinic appointments Mean (Range)	 3.2 (0–12)	 3.5 (0–8)	 3.7 (0–6)	 =0.7
Change in VA (ETDRS letters)	-4.7	-5.7	-4.8	=0.9
Other ocular problems	2 glaucoma	8 glaucoma	1 CRVO	
Mean number of anti HTN drugs	2.3	1.9	1.8	=0.2
Anti DM drugs Oral + Insulin Insulin Oral only	 20 11 6	 26 9 9	 8 5 3	
Baseline HbA1C	8.87	9.5	8.6	=0.15
Baseline Sys BP	140	148	138	=0.16
Baseline Dias BP	80	83	72	=0.003
Baseline BMI	29.7	29.7	27.3	=0.2

Table 2.6 a: Univariate analysis of the prognostic systemic and ocular factors for gain in vision after macular laser treatment for DMO

SYSTEMIC FACTORS	**Gainers (n = 16)**	**Losers (n = 37)**	**Odds ratio (95% CI)**	***P*** **(Fisher'sexact)**
Age at baseline (years)				
<60	8	13	1.8	0.4
≥60	8	24	(0.5-6)	
Ethnic groups				
Caucasians	4	10	0.9	1
Non-Caucasians	12	27	(0.2-3.4)	
Gender				
Male	12	19	2.8	0.1
Female	4	18	(0.7-10.4)	
Duration of diabetes				
(years)	10	26	0.7	0.7
< 15	6	11	(0.2-2.4	
≥ 15				
Diabetic medications				
Oral	6	3	6.8	0.01
Insulin/ oral +insulin	10	34	(1.4-32.2)	
Baseline HbA1C				
< 7.5	5	8	1.64	0.5
≥ 7.5	11	29	(0.4-6.1)	
Baseline systolic BP				
< 140	4	16	0.4	0.2
≥ 140	12	21	(0.1-1.6)	
Baseline diastolic BP				
< 100	13	34	0.4	0.3
≥ 100	3	3	(0.06-2.1)	
No: anti-hypertensives at end of follow-up				
0–2	4	23	4.9	0.01
≥ 3	12	14	(1.3-18.3)	
Baseline BMI				
< 30	7	29	0.2	0.02
≥ 30	9	8	(0.06-0.7)	

Table 2.6 b: Univariate analysis of the prognostic systemic and ocular factors for gain in vision after macular laser treatment for DMO.

OCULAR FACTORS	**Gainers (n = 16)**	**Losers (n = 37)**	**Odds ratio (95% CI)**	***P* value**
Baseline VA (ETDRS letters)				
< 60	10	8	6.04	0.01
≥ 60	6	29	(1.6-21)	
Lens status:				
Phakic	44	48		0.2
Prior pseudophakia	3	5		
Pseudophakia during study	2	5		
DR status at baseline				
Non-PDR	15	32	0.4	0.6
PDR	1	5	(0.04-3.9)	
Number of macular laser treatments				
1–3	7	16	1.02	1
> 3	9	21	(0.3-3.3)	
Number of clinic appointments	19.23	19.6		
mean	4–34	3–39		1
range				
Number of failed clinic appointments	3.02	3.75		0.009
Mean	0–7	0–12		
Range				

Gainers: gain of 10 or more ETDRS letters; Losers: loss of 10 or more ETDRS letters. Abbreviations: PRP: pan-retinal photocoagulation; ETDRS: early treatment diabetic retinopathy study; HT: hypertension; DM: diabetes mellitus; BMI: body mass index

Table 2.7: Multiple regression model for visual outcome at 5 years

INDEPENDENT VARIABLES	Coefficient	Std. Error	r	t	P
(Constant)	-7.2531				
Dias_BP_baseline	0.7058	0.2614	0.3664	2.700	0.0096
Diff_in_dias_BP	0.8148	0.3085	0.3595	2.641	0.0112
Diff_in_HbA1c	2.3180	1.1078	0.2919	2.092	0.0418
Diff_in_syst_BP	-0.1791	0.1065	-0.2381	-1.681	0.0994
VA_at_baseline__ pre_laser_	-0.8700	0.1714	-0.5951	-5.076	<0.0001

BMI: body mass index; VA: visual acuity; CI: confidence interval.

2.1.5 Discussion

The aim was to test the hypothesis that VA may continue to improve in eyes with laser-treated maculopathy (H1). I investigated the long-term (5-year) outcome of laser photocoagulation in a real-life, inner-city population. I was successful in recruiting 100 subjects who were followed-up for 5years after their initial laser treatment for diabetic maculopathy. The mean annual visual outcomes and systemic parameters were collected retrospectively and compared to outcomes of the laser arm of the DRCRN trial in this section. The mean change in VA at 5 years was found to be -5.23, with the 3-year outcome being inferior to the clinical trial results, with more people gaining vision (≥15 letter gain) in the DRCRN group compared to this cohort (26% versus 9%)(section 2.1). Furthermore, 3 times more patients lost vision (>15 letter loss) in the real-life setting of this cohort compared to the clinical trial results of the DRCRN group (27% versus 8%, respectively). These results suggest that laser treatment is only effective at halting—and not reversing—retinal deterioration. It can be argued that these patients are not trial patients and so not standardized with regard to age; race; sex; diabetic control; other associated systemic conditions, but the outcome suggests a real life scenario, where there is no standardization of the patients. The study has adequate power (sample size calculated for 95% confidence), regular follow-up for 5years, availability of visual acuities for all the visits, and other systemic functions including blood pressure, blood glucose levels for each visit. Visual acuities were tested by Snellen charts according to clinic protocols and were later converted to logmar for data processing. Errors may therefore have occurred whilst assessing vision, and to help compensate for these variations, we considered annual mean visual acuities.

This long-term laser outcome study (chapter 2) assessed a number of factors that may determine the poorer outcome. The VA examiners were not certified, and VA measurements were recorded in busy clinic settings, patients weren't tested with their updated refraction. Also under these circumstances, it is possible that the examiner did not spend enough time encouraging the patient to read as far as possible. As a result, the best-corrected VA (BCVA) may have been underestimated at times. Further overall discussion and the attributes and deficits of the study are carried out in chapters 4 & 5.

2.2 Prevention of dark adaptation causes regression of diabetic macular oedema

2.2.1 Introduction:

The understanding of pathophysiology of DMO is still ill understood. Though it is generally considered that damage caused by reactive oxygen species is central (Kowluru RA, 2007; Brownlee M, 2005), recent reports that intravitreal injection of anti-VEGF drugs improves diabetic maculopathy (Elman MJ, 2012; Massin P, 2010; Boscia F, 2010; Nguyen QD, 2009, 2010; Shetty R, 2008; Kumar A, 2007; Solaiman KA, 2010; Funk M, 2010; Goyal S, 2010; Michaelides M, 2010) suggest alternative mechanisms for formation of DMO. A finding which supports this hypothesis is that retinal endothelial cells in culture do not show signs of oxidative stress when exposed to high concentrations of glucose (Busik JV, 2008). In addition, there are other findings that are difficult to reconcile with the idea that oxidative stress is the primary cause of DMO (Brownlee M, 2005; Arden GB, 2011).

Another school of thought is that the large demand of dark adapted rods for oxygen is important in pathogenesis of DMO, and a proposed mechanism is that up-regulation of VEGF, which is induced by retinal hypoxia (Aiello LP, 1995; Miller JW, 2012) can

directly and adversely damage small vessels, which would reduce local capillary blood flow and increase hypoxia in a positive feedback cycle (Arden GB, 1998, 1999, 2001, 2004, 2005). There are indirect evidences that support this view (Nguyen QD, 2004; Kern TS, 1996; Shiba T, 2009; West SD, 2010). Nguyen QD et al confirmed that breathing 100% oxygen reverses contrast sensitivity and vascular perfusion changes in patients with minimal maculopathy suggesting hypoxia may also contribute to functional changes in pre-retinopathy stage of the disease (Nguyen QD, 2004). Because the oxygen demand of rods is very much increased in darkness (Hagins WA, 1989; Hamer RD, 2005; Braun RD, 1995; Linsenmeier RA, 1998; Birol G, 2007), it was hypothesised that prevention of dark adaptation should reduce the rate of progression of DMO. Most of the dark adaptation occurs during sleep. Therefore, by sleeping under conditions in which there is sufficient light to maintain the rods in a semi–light adapted state, decreasing the oxygen consumption might reduce DMO. A phase I clinical trial involving 12 patients with mild background DR showed that trans–lid illumination of the retina during sleep was acceptable by patients, who reported no ill effects and showed improved retinal appearance and function (Arden GB, 2010; Jyothi S, 2010; Arden GB, 2010). In light of this finding, a further investigation (Phase II trial) was conducted to provide "proof of principle" for this concept. This trial was conducted in accordance with the Declaration of Helsinki and guidelines on good clinical practice. Approval was obtained from King's College Hospital Ethics Committee (ISRCTN34037927; R&D 08/H0808/198)(approval letter in appendix Chapter 7). Written informed consent was obtained from all participants.

2.2.2 Methods:

2.2.2.1 Participants:

Patients for this study were recruited from diabetic clinics at "Laser and Retinal Research Unit of King's College Hospital, London" that assessed the effect of light therapy for diabetic maculopathy. Patients were all referred to the Medical Retina Department of KCH by the DR screening service, as a result of grading of standard 2-field fundus photographs (Harding S, 2003). Diabetic maculopathy on 2-field colour photography was defined as the presence of exudate within 1 DD of the centre of the fovea, circinate, or group of exudates within the macula, retinal thickening within 1 DD of the centre of the fovea (if stereo available), or any microaneurysm or haemorrhage within 1 DD of the centre of the fovea only if associated with a best VA of $\leq$ 6/12. Eyes with clinically significant DMO that were eligible for laser treatment were excluded. Those with clinical evidence of focal macular thickening and those with small "dark retinal anomalies" very near the fovea in at least one eye were recruited. This group, defined as non-significant macular oedema (M1), consisted of persons who were much less severely affected than those recruits for recent trials of anti-VEGF agents for DMO (Massin P, 2010; Boscia F, 2010; Nguyen QD, 2009, 2010; DRCRN, 2010; Shetty R, 2008; Kumar A, 2007; Solaiman KA, 2010; Funk M, 2010; Goyal S, 2010; Michaelides M, 2010). Patients in the M1 group are those who are not usually treated immediately, but who are watched until the condition progresses, when laser treatment is offered.

All study participants provided informed consent for the clinical trial. Patients underwent a full ophthalmic examination, including objective and subjective refraction, contrast and colour contrast sensitivity, microperimetry, and slit-lamp biomicroscopy

and OCT at baseline, 3, and 6 months. Glycosylated haemoglobin was also estimated at baseline visit and at 6 months reviews at the hospital laboratory.

The study was designed to test the primary hypothesis that the light-masks would delay or reverse the progression of DMO. Participants met all of the following entry criteria in at least 1 eye: (1) exudates or thickening within 2 optic DD of the centre of the macula (1 optic DD is defined as 1500 μm) graded on 2-field photography, one centred on the optic disc and the other on the macula; (2) no history of scatter (pan-retinal) or focal/grid photocoagulation for DR; and (3) no evidence of any other ocular pathology that could interfere with ocular examination and VA assessment during the 6-month study period.

Participants were excluded if they had: (1) clinically significant DMO, that was amenable to laser photocoagulation at baseline according to ETDRS guidelines; (2) highly asymmetric grades of DR between eyes; (3) VA reduction that could not be attributable to DR; (4) any other concomitant ocular or systemic condition that could influence the natural course of DR.

The eye of each participant that met all of the inclusion criteria was designated as the "study eye," and a light-mask was applied during the sleeping hours for the period of the study.

2.2.2.2 Construction and design of light-masks:

Light was provided by four light-emitting diodes (Nichea NESE021T rank E) of 1 × 1 mm in diameter, surface-mounted 5-mm apart in a square array on a thin, flexible, printed circuit board. Diodes were encapsulated in a plastic cup of transparent silicone rubber, of a quality certified as medically inert. The concave surface of the cup fitted on

the closed lid, thus centring the light in the optic axis. The diodes were fitted to either the left or right eye, not both. The flexible circuit board was joined to a rigid board that contained the driving electronics.

The peak light output was at 505 nm, which was chosen to maximise the preferential light absorption by rods, rather than cones. In preliminary results (not reported) that were similar to those described before (Arden GB, 1999), we determined that the light used increased the rod threshold by 3-4 log units before the increment threshold for cones increased. (Arden GB, 2010)

The device was driven by a 3v (nominal) rechargeable battery (Panasonic UL23300), of 50 mA h capacity. It was connected to a charge pump chip (Maxim 1573) that stabilised the battery voltage and also provided four independent drivers for the LEDs. A multivibrator circuit produced a train of short light-pulses, the mark-space ratio of which could be altered to regulate the light output. Each LED drew approximately 200 µA current. The light output remained nearly constant for >50 h without recharging the battery. Patients were instructed to recharge the battery each morning, by placing the unit on a box that contained the primary of an induction charger. The secondary was on the light-mask printed circuit board, which also contained a rectifying circuit. A relay on the circuit board was activated when the mask was placed on the box, and when the mask was positioned correctly; the light from the diodes gets turned off. Masks were covered in a plasticized thick cotton cover (the plastic was inert) such that no electrical part was exposed (**Figure** 2.3). This cover was held against the eyes by an elastic headband. The light output intensity of 505 nm light required causing rod semi saturation was established in the Phase I trial (Arden GB, 2010).

Figure 2.3: light mask with LED lights

The design had 2 faults that could influence the results. One was that, in use, the electronics failed in around half the units; therefore, many patients had interruptions of a few days in their treatment (maximum 7 d; range 3-7 d). The second was that the plastic cup could slip from the central position whilst asleep; in such a case, retinal illumination was reduced to an unknown extent.

2.2.2.3 Study design:

The study was at a single centre and was single-masked (optometrists and photographers were masked of the study eye). The chosen eye to be illuminated was always the worst. In many cases, the fellow eye showed minimal changes of DMO, as reported below. Eligible patients were provided with the information leaflet and allowed to use the eye mask in one eye for a week before consenting to the trial. They were screened if they agreed to continue. Following this baseline first visit, patients returned after 3 and 6 months. Reasons for dropout were noted. Patients were contacted between visits to ensure compliance and encouraged to return to the trial centre if they had any trouble with the masks (electrical failures occurred in 30% of masks). Patients were questioned about any sleep disturbance, but formal questionnaires were not used. An ophthalmologic examination was performed at baseline, 3, and 6 months. All tests were

carried out according to standardized protocols by optometrists and photographers who were accredited for clinical trials. None of the patients had any previous experience of performing the tests.

2.2.2.4 Refraction:

Overcorrection or undercorrection of refraction can lead to assessment errors; therefore, assessment distances for various charts were followed strictly according to the manufacturers' protocols, and corrections were made to the prescription accordingly to maintain repeatability of vision (Blackhurst, 1989). A certified optician performed non-cycloplegic subjective refraction if updated prescription was not available. ETDRS chart R was used for refraction. If the subjective refraction agreed with the prescription of the patient, then they were allowed to wear their prescription glasses. During the tests, which were done from nearer distances, the prescription of the patient was appropriately corrected for distance (i.e., +0.75 was added for tests done from a 1-m distance). This situation best reflects the true clinical trial setting, in which many measurements are taken by several observers according to set protocols, but often without formal assessment of intersession repeatability.

2.2.2.5 Vision:

The VA was measured with ETDRS charts at 4-m distance as described in the earlier sections (1.5.3.1). This procedure was repeated at the 3- and 6-month follow-ups visits by a certified research optician.

2.2.2.6 Optical coherence tomography:

Scanning with the Cirrus HD-OCT (Cirrus, Carl Zeiss Meditec AG, Jena, Germany) was performed with the 512 × 128 scan pattern, in which a 6 mm × 6 mm macular grid was scanned with 128 horizontal B-scan lines, each consisting of 512 A-scans per line (total of 65,536 sampled points) as described in chapter 1 (section 1.5.2.2). The central subfield thickness was used for comparison. **Figure** 2.4 shows the OCT scan of foveal region.

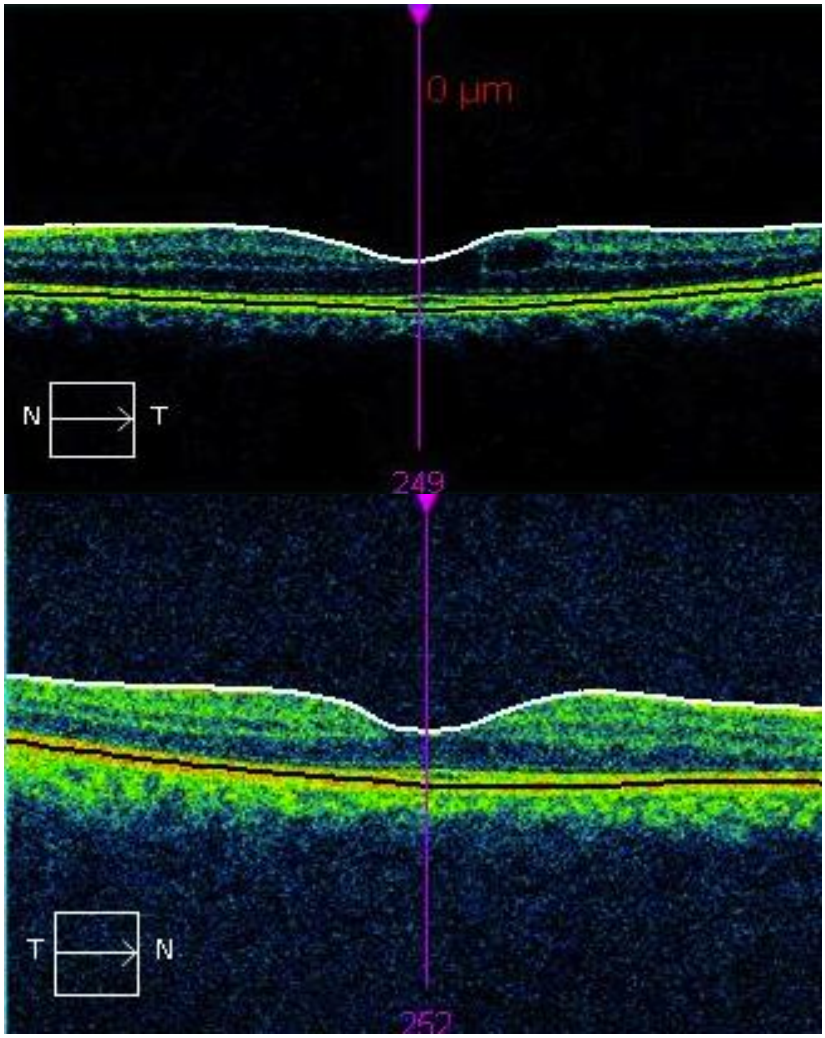

Figure 2.4 Morphological appearance of parafoveal intraretinal cyst on OCT (top image) compared to a normal OCT (bottom image).

2.2.2.7 Chroma test:

The test was exclusively carried out by the author (SJ) to a standardised protocol as described in section 1.5.3.2 of chapter 1. The diabetic module of the Chroma test was used for assessment. The protan and tritan retinal sensitivity thresholds were noted (Wong, 2008.). The test was exclusively carried out by the author (SJ) to a standardised protocol. With undilated eyes, colour contrast sensitivity was tested in each eye monocularly by using the diabetic module of the Chroma test software program. With appropriate refractive correction, the subject was seated at a fixed distance of 1 m from the monitor, so that the alphabetical letter displayed on the computer screen subtends a constant angle on the retina. The letter size creates an image that tests the central 6.5° of the retina. Letters were displayed on a background of equiluminance. Luminance of the screen was calibrated before the start of each session. The computer finds the endpoint of the test by a Modified Binary Search method. The protan and tritan retinal sensitivity thresholds were noted (Wong, 2008.).

2.2.2.8 Contrast sensitivity (CS):

After corrective refraction (with +0.75 addition), monocular CS was assessed by a standardized protocol with the Pelli-Robson chart (Clement Clarke Inc., Columbus, OH). Different charts were used for the left and right eyes, at a distance of 1 m and chart luminance of 80–120 cd/m^2.

2.2.2.9 Nidek microperimetry 1:

Microperimetry was performed with the Nidek MP1 microperimeter (MP1 Nidek Technologies, Japan).

2.2.2.10 Fundus photograph:

Fundus stereographic pictures were taken with the Topcon TRC 50IX mydriatic fundus camera. Images were captured on the posterior pole at 50° and on the fovea at 20°. Two observers graded the OCT and fundus photographic images. Kappa statistics of interobserver agreement showed good agreement (K=0.8).

2.2.2.11 Decision to treat:

Decisions regarding application of macular laser photocoagulation resided with the principal investigator, but laser was applied only if the retinal thickening progressed to within 500 µm of the centre of the macula, with no angiographic evidence of macular ischaemia. Pan-retinal photocoagulation for DR was initiated at the investigator's discretion, but it was expected that it would not be applied before development of retinal or optic disc neovascularisation (proliferative DR defined as ETDRS level ≥ 65).

2.2.2.12 Safety assessments:

Appropriate channels were ensured in place to report any adverse events.

2.2.3 Statistical analyses

We performed statistical analyses using 2-tailed paired *t*-test to explore changes in CST, AMT, total macular volume, VA, contrast sensitivity, tritan and protan threshold, and mean retinal sensitivity at 3 and 6 months after starting treatment. The change in individual eyes varied considerably and was not normally distributed (SD > mean). Therefore, eyes were classified as "improved", "unchanged", or "deteriorated". The binomial probabilities of the numbers changed were calculated (**Table** 2.8) by assuming that the probability of improvement or deterioration was equal to 0.5. This assumption

is very conservative, because most patients would continue to deteriorate if untreated. An estimate of the true probabilities and the consequence is given in the text. The Fisher exact test was used to compare categorical outcomes where appropriate. All analyses were carried out after the end of the trial. The null hypothesis was rejected for $P < 0.05$.

2.2.4 Results:

The trial enrolled 40 patients, 5 were with Type I DM (12%) and the remainder with Type II DM. Ages ranged 33–75 years (mean: 56 years). Glycated haemoglobin (HbA1C) levels ranged 6.0-11.6% (mean: 8.21% at baseline). The mean change in HbA1C at 6 months was -0.17 ± 0.88.

2.2.4.1 Dropouts:

Out of the 40 individuals recruited for the study, light-masks were not given to 5 individuals at baseline, due to defective masks. Three patients failed to complete the study; one due to ill health, one withdrew after 3 months due to inability to keep hospital appointments, and one failed to use the light-mask. A total of 65 eyes were included for baseline studies and analyses of changes at 3 and 6 months (65 eyes studied out of 70 eyes; 5 non trial eyes were excluded because of planned interventions or already had intervention). Inter eye comparisons were not done in patients who received laser/intravitreal bevacizumab for progression of DMO in the fellow eyes during the study. None of the illuminated eyes required such treatment during the trial. Of the 35 trial eyes followed-up for 6 months, only 27 eyes had microperimetry results at 3 months (7 eyes couldn't be completed). At the end of 6 months visits 34 trial eyes had microperimetry available for assessing progression (MP1 on one eye couldn't be completed). Data for only twenty eyes were available in non-trial eyes at 3rd and 6th

month follow-up for microperimetry for inter eye comparison as others found this test too difficult and did not want to repeat the test in non trial eyes after their baseline visits.

2.2.4.2 Adverse effects:

None of the recruited subjects reported mood alteration or any difficulty in wearing the masks or sleeping.

2.2.4.3 Morphological changes on OCT:

Figure 2.5 shows nonparametric assessment by OCT images representative of the findings in the treated eyes. Areas of thickened retina decreased during the period of the trial and the average macular thickness returned toward normal. In the cross-sectional image, diminution of cysts can be seen. The OCT images were classified into three groups: those in which the DMO regressed, those in which it advanced, and those in which no definite change could be observed. The number of cases in each group is shown in **Table** 2.8. Trial and fellow eyes differed considerably in the proportion with cysts at baseline recruitment. In addition, the degree of swelling was greater in almost all trial eyes than in the corresponding fellow eye ($p = 0.0015$). In trial eyes, 27 out of 35 had baseline cysts, whereas in fellow eyes only 8 had baseline cysts out of 30 eyes.

A number of trial eyes were judged to have improved during the trial. Diabetic eye disease is usually progressive; if it were stationary, then the probability that improvement would be (erroneously) detected would be equal to the probability that the eye would be considered to have deteriorated. In such a case, the binomial probability of obtaining the result is small, as indicated in **Table** 2.8, both for changes in cyst size

(1) and overall area of abnormal swelling (2). If the cases scored as “no change” are assigned equally to the 2 groups, then the probabilities remain low.

Table 2.8: Changes in OCT appearances from beginning to end of the trial. Improvement and worsening is determined based on consensual agreement between two independent observers. In case of disagreement, an arbitration was taken from a third independent observer.

	Trial eyes with cysts	**Fellow eyes with cysts**
TOTAL	27	8
Improved	19	1
Worsened	4	3
No change	4	4
	Overall grading	Overall grading
Improved	19	3
Worsened	7	3
No change	9	24

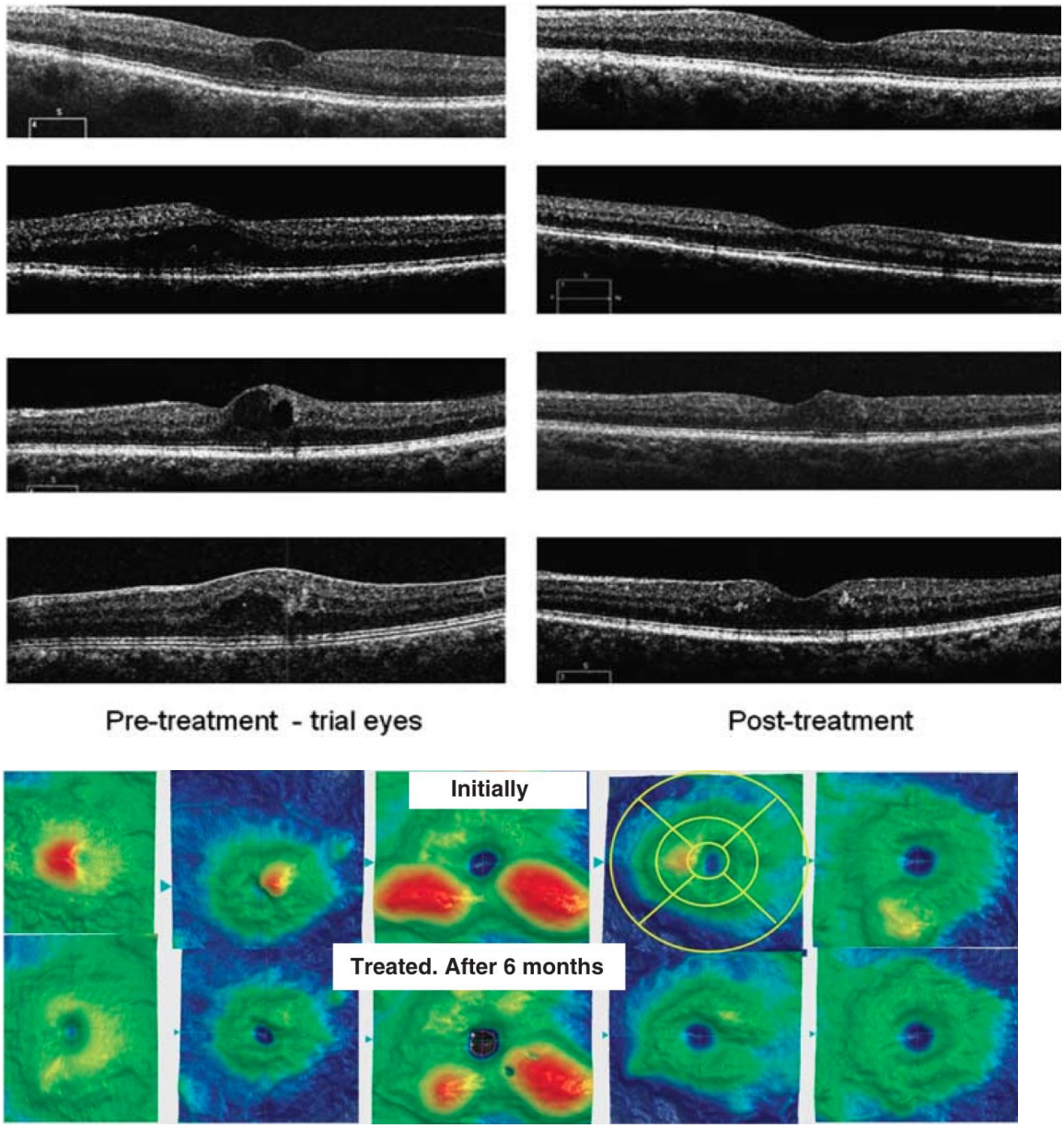

Figure 2.5: OCT changes over 6 months. a) cross section raster images through the fovea depicting pre and post treatment cyst changes. b) Computed 3-D OCT false colour images in representative patients, showing changes in retinal thickness. Blue and dark green colours are within normal limits, and green, yellow, red, pink, and white represent regions of increased thickness, which varies between patients. Swelling diminishes during the trial. Quantitation of such results was achieved by using the separation of different zones, included in the OCT report, which are indicated for one eye, in the figure. These are based on the ETDRS zones, and results are given in the **Table** 2.9. Zone 1 is the subfoveal zone for the left-handed patient, the zone measured was zone 2, and measurements are also presented from zone 4, opposite it, which is called the 'mirror zone'. For the right-most pair of images, measurements would be taken from zone 5 and its mirror zone, ETDRS zone 3.

2.2.4.4 Quantitative comparisons:

The area of retinal swelling associated with early DMO was, in our patients, highly localised (**Figure** 2.5). Therefore, the average change in retinal thickness or volume in the entire area under investigation, which is a criterion used in other trials with more advanced disease (Massin P, 2010; Boscia F, 2010; Michaelides M, 2010), was inappropriate.

We recorded retinal thickness in the central subfield (1) and in the zone where thickness was greatest. This location was always in the intermediate zones, 2, 3, 4, or 5, and varied from patient to patient. A third measurement was made in the zone at 180° from the most thickened zone, where the swelling was usually much less or even absent (**Figure** 2.6). **Table** 2.9 shows the means and variation in thickness of the patients' retinas at the initial measurement. These values were compared with the similar values after 6 months.

Table 2.9: Changes in OCT average macular thickness measurements (ILM-RPE)

	Trial eyes with cysts			**Fellow eyes with cysts**		
TOTAL (μ)	Zone 1	Worst zone	Zone opposite to worst zone	Zone 1	Worst zone	Zone opposite to worst zone
Initial	273±9.3	338±4.6	315±3.5	257±11.4	334±6.9	309±5.6
6m	268±9.3	328±4.9	315±3.2	252±7.2	324±5.3	305±5.5
P value	0.2	0.007	0.8	0.7	0.09	0.4

ILM: Internal limiting membrane; RPE: retinal pigment epithelium

All 3 zones included in **Table** 2.9 were slightly thickened, both in comparison to machine normal values and in comparison to fellow eyes. The central subfield thickening was slightly and insignificantly reduced during the course of the trial. In the most thickened zone (2, 3, 4, or 5), the mean retinal thickness significantly decreased by 10 mm during the course of the trial. For the opposite zones, the ILM to RPE depth did not change during the trial. There were no significant changes in the untreated eyes.

The zone of maximum swelling (2, 3, 4, or 5) decreased by an average value of 12 mm over 6 months. The zone at 180° relative to the swollen zone showed no change. The reduction in swelling was significantly different from zero. In almost all patients, intermediary OCT examinations were made, and differences between 1 and 3 months and between 3 and 6 months were calculated; these values were significant only for the zone of maximum swelling. When results from untreated eyes were examined, changes in thickness were all insignificant. Therefore, instrumental errors cannot account for shrinkage in trial eyes.

The shrinkage differed remarkably in different patients; the distribution is shown in **Figure** 2.5 for the zone of maximum swelling. Similar results were calculated for the other zones of the treated eyes and were not significant. In the few cases in which the untreated eyes changed during the trial, the number in which the oedema grew worse was larger than the number of improvements (**Table** 2.8). Fundus photographs were graded with standard photographs according to the National Screening Grading system. No significant change in severity of retinopathy occurred during the course of the trial.

2.2.4.5 Psychophysical testing:

The summary of the results for colour vision, VA, and achromatic contrast sensitivity testing were given in **Table** 2.10. In the trial eyes, changes in protan sensitivity were not significant, but the tritan threshold decrease and the increase in the number of ETDRS letter and Pelli-Robson letters were significant. The average final VA > 80 letters read corresponds to a value that is slightly better than 20/20 or decimal 1.0. During the same period of time, the VA of the control eyes fell slightly but significantly. In the second half of the trial, the only significant fall in treated eyes was in the tritan threshold.

Table 2.11 summarise the results from microperimetry. Sensitivity of the central area and peripheral zones of the treated eyes increased, and the number of patients showing improvements increased during the second half of the trial. Changes obtained from untreated eyes were smaller and inconsistent. There was no significant difference between the initial microperimetry results of the 2 groups.

Table 2.10: Summary of psychophysical tests

	Trial eyes				**Fellow eyes**			
Test	Protan	Tritan	ETDRS	Contrast	Protan	Tritan	ETDRS	Contrast
Initial (mean)	7.5	28.2	78.3	31.2	8.8	29.4	76.4	31.7
6m (mean)	6.5	22.4	80.7	33.3	7.7	26	74.1	30.8
No: improved	20	22	16	16	11	17	10	14
No: deterioratd	9	8	7	6	17	10	18	15
P binomial	0.01	0.005	0.02	0.01	0.08	0.06	0.04	0.14
P value	0.10	0.17	0.11	0.09	0.05	0.21	0.23	0.34
T-test between trial and control eyes (P value)					0.95	0.75	0.05	0.01

Table 2.11: Microperimetry: dB attenuation for threshold

	Central zone (zone 1)		Mean of zones 2-9	
	Initially	3 months later	Initially	3 months later
Number	35	27	35	27
Mean ± SE	13.2 ± 0.6	15.5 ± 0.5	13.3 ± 0.5	15.7 ± 0.5
Number improved		14		16
Number worsened		5		4
Binomial probability		0.0187		0.0001
Paired t-test		0.0030		0.0008

2.2.4.6 Summary of results:

In the eye exposed to light during sleep, reversal of early morphological changes occurred: cysts and generalised oedema were markedly reduced and, in local areas of maximum swelling, the decrease was significant. In untreated eyes, no such changes occurred.

This relatively small study has provided evidence that in patients with very early DMO, significant local morphological and functional improvements occur in the eye exposed to light during sleep. In untreated eyes, any morphological change is a deterioration. The natural progression of the diabetic maculopathy is generally to deteriorate, but in treated eyes the cystic changes disappeared whilst no new lesions developed in contrast to non treated eyes. Standard statistical methods thus show that the changes associated with treatment are unlikely to be due to chance. The visual acuity improvement was not statistically significant when treated eyes were compared to non treated eyes because of "ceiling effect", since most of the recruited eyes have only early maculopathy and as it

is, the visual acuities were near normal. The study is limited by lack of double masking and randomization, not a robust design of the mask, which can ensure proper placement of the light source in papillary axis.

Even if all the electricity produced by the source were converted into photon energy, the resultant retinal illumination would be 1 mW/cm^2. Such minimal intensities wouldn't cause any retinal damage nor cause any disturbances of diurnal rhythm. The retinal illumination (480 nm) required to cause a 50% reduction in the night-time increase of melatonin is 25 mW/cm^2 (Young RW, 1971). Further large-scale fully randomised and controlled clinical trials are required to establish the efficacy of this method of treatment, the quantity of light required, the long-term effects.

3: DIAGNOSING EARLY DIABETIC MACULOPATHY

3.1 Short-term changes in visual function and macular thickness in patients with no or minimal diabetic retinopathy

3.1.1 Introduction:

Diabetic retinopathy is defined and graded by the presence of clinically visible retinal vascular changes. However, there is sufficient evidence that neurodegenerative changes precede or accompany these vascular changes in histological examinations of the retina (Barber AJ, 1998). Furthermore, changes in visual function occur before the onset of any clinically visible vascular changes in the macula (Westall CA, 2005; Lopes de Faria JM, 2001; Brinchmann-Hansen O, 1993; Lieth E, 2000). The relationship between the neuronal and vascular changes is complex (Chen FK, 2011; Kube T, 2005; Okada K, 2006; Rohrschneider K, 2008; Vujosevic S, 2006; Trick GL, 1988; Alkuraya H, 1989; Brinchmann Hansen O, 1993) and a varying correlation was found between structural and functional assessments (Chen, 2011; Kube, 2005; Okada, 2006; Rohrschneider, 2008; Vujosevic, 2006; Trick, 1988; Alkuraya, 1989; Brinchmann Hansen, 1993). The relative rate of progression of the neuronal and vascular components also remains unclear. The processes involved with cell-death can have a slow and insidious course and so short-term studies on visual function changes are unlikely to reflect the rate of neuronal cell death. However, several investigators have demonstrated short-term or acute changes in retinal neuronal function in diabetes. Shirao and Kawasaki showed that oscillatory potentials that originate in the amacrine cells are prolonged in the pre-retinopathy stage and this response correlates to the acute deterioration of non-photic electro-oculogram to intravenous 7% sodium bicarbonate in these patients (Shirao Y, 1998). These electro-oculogram changes suggest retinal pigment epitheliopathy in the

pre-retinopathy patient. Similarly, breathing 100% oxygen reverses contrast sensitivity and vascular perfusion changes in patients with minimal retinopathy suggesting hypoxia may also contribute to functional changes in pre-retinopathy stage of the disease (Nguyen QD, 2004; Dean FM, 1997). Other changes including loss of synaptic proteins, changes in intracellular signalling and extracellular glutamate activity that signify retinal neuronal dysfunction have also been reported in diabetic animals (Lorenzi M, 2001). So, in order to assist with clinical decision-making process, it is important to differentiate between a change criterion induced by real short-term biological changes due to diabetes and within-subject variability (measurement error). (Hypothesis H3)

The purpose of this part of the study was to determine whether patients with no or very minimal retinopathy demonstrate changes in visual function over 6 months despite no detectable changes in retinal vasculature or blood sugar levels. We then compared the changes to published data on the repeatability of each visual function to establish whether the change criterion detected could be considered as genuine beyond measurement errors.

3.1.2. Materials and methods:

The data of contralateral eyes of subjects recruited to a clinical trial (ISRCTN34037927) was analysed retrospectively. The clinical trial assessed the effect of a specially designed light mask for the treatment of early maculopthy in one eye for 6 months. The patients were reviewed at 3 and 6 months. All the contralateral eyes with no or early diabetic retinopathy, with no maculopathy defined as central subfield thickness (CST) <308 µm on Cirrus OCT at all 3 visits were included. This ensured that all anatomical changes in the central subfield were within the normative range of central

subfield thickness on Cirrus OCT. Excluded were those with ocular co-morbidity and those with central subfield thickness of ≥ 308 μm at any visit or those who had previous treatment for diabetic eye disease. The study adhered to tenets of the Declaration of Helsinki for research involving human subjects and was approved by Kings College Hospital Ethics Committee (R&D 08/H0808/198, appendix 7). Each patient gave written consent before enrolment and for undergoing these tests as part of the clinical trial.

All patients underwent visual function assessments including best-corrected visual acuity, achromatic and chromatic contrast sensitivity, retinal sensitivity measured by microperimetry at baseline, 3 and 6 months. All tests were carried out according to the standardized protocols and carried out by optometrists and photographers accredited for clinical trials. None of these patients had any previous experience of performing these tests.

3.1.2.1 Visual acuity:

Best-corrected visual acuity measurement was performed with Early Treatment of Diabetic Retinopathy Study (ETDRS) charts at 4 m. The ETDRS charts are modified versions of the logarithm of the minimum angle of resolution (logMAR) charts designed by Bailey and Lovie (Lighthouse International, New York, NY). Previous studies have tested the repeatability of scores on these charts. Reported estimates of the 95% coefficient of repeatability of the ETDRS charts ranged from 3.5 to 8 letters in normal subjects (Elliott DB, 1988; Raasch TW, 1998)

3.1.2.2 Contrast sensitivity:

Contrast sensitivity was assessed as total letters scored with the Pelli-Robson contrast sensitivity chart (Haag-Streit USA, Mason, Ohio, USA). The details of the procedure were as discussed in chapter 1 (section 1.5.3.3). Reported estimates of the 95% coefficient of repeatability of the Pelli-Robson charts ranged from 3-6 letters in normal subjects (Lovie-Kitchin JE, 2000; Elliott DB, 1990; Dougherty BE, 2005; Elliott DB, 1993).

3.1.2.3 Colour sensitivity:

The colour vision thresholds were determined by the Chroma test. The colour contrast sensitivity was tested in each eye monocularly using the diabetic module of Chroma test, software programme analyzing the age-corrected tritan (TCCT) and protan colour contrast thresholds (PCCT) (Wong R, 2008). The computer finds the endpoint of the test by a Modified Binary Search method. The retinal sensitivity thresholds were noted.

3.1.2.4 Retinal threshold by Microperimetry:

The Nidek Microperimeter was used to quantify macular sensitivity. We used a fast threshold strategy to reduce the test time and influence of fatigability on the results. The test–retest variability of mean macular and point-wise retinal sensitivity has been investigated before in small samples of normal individuals (Hwang JC, 2005). They found that the averaged difference between tests for the 40 locations ranged between 0.1 and 1.6 dB.

3.1.2.5 Optical Coherence Tomogram (OCT):

Spectral-domain OCT was performed using the Macular Cube 512 x 128 scanning protocols (Cirrus, Carl Zeiss Meditec AG, Jena, Germany). The in-built software calculates average macular thickness (AMT) of all the 9 ETDRS-like zones, the central subfield thickness (CST) and the macular volume. Two observers graded the OCT and fundus photographic images for any morphological changes at the macula at all 3 visits. Only eyes with no morphological alteration and central subfield thickness of less than 308 µm in all 3 visits were included in this study. Measurements of central subfield thickness are repeatable and reproducible in normal eyes with no macular pathology. The inter rater repeatability coefficients obtained measuring the central macular subfield was 10.2 µm for Cirrus HD-OCT in normal eyes (Giammaria D, 2011).

A blood sample was also obtained from each participant at baseline visit and at month 6 to measure glycosylated haemoglobin (HbA1c) by the hospital clinical laboratory.

3.1.3.Statistical Analysis:

Med cal version 11.4 was used for the statistical calculations. The standard error of measurement (SEM) represents the extent to which a variable can vary in a measurement process. The Standard errors of measurement (Domholdt E, 2005) were calculated using the following equation: $\mathrm{SEM} = sd \times \sqrt{(1 - r)}$. In this equation, ***sd*** is the standard deviation of the measure, and ***r*** is the reliability coefficient (test-retest reliability in the form of intraclass correlation coefficient (ICC) for the subject group).

3.1.4 Results:

Thirty-five eyes of 35 patients met the inclusion-exclusion criteria for this post-hoc analysis. The mean age of the patients was 55.9 yr. The mean change of HbA1c for the time of follow-up was negligible (p=0.39). The OCT scans of all the eyes were assessed by two independent assessors and agreed in all cases that no clinically visible morphological progression was noted in 6 months of follow up and the central subfield thickness in all these cases in all visits were less than 308 µm. **Table** 3.1 shows the mean values of the visual indices at baseline and 6 months.

Table 3.1: Mean values of the visual indices at baseline and 6 months

	Initial value			Final value (6 months)			Difference			95% CI	p
	Mean	SD	SE Mean	Mean	SD	SE Mean	Mean	SD	SE Mean		
BCVA	77.7	±9	1.8	75	±8.3	1.6	-3.3	±10	2.1	-7.6 to 0.9	0.1
Contrast sensitivity	32.5	±5.2	1.05	30.8	±4.2	0.8	-1.8	±5.0	1.0	-3.8 to 0.2	0.08
Protan threshold	6.7	±4.2	0.8	7	±4.7	0.9	0.2	±3	0.6	-0.9 to 1.5	0.6
Tritan Threshold	24.7	±23.7	4.7	22.7	±22	4.4	-2.1	±11.3	2.3	-6.7 to 2.5	0.3
Mean macular sensitivity	14.5	±3.5	0.7	15	±4.1	0.8	-0.2	±2.4	0.5	-1.4 to 1.0	0.7
Mean foveal sensitivity	14.3	±4.2	0.8	14.6	±4.4	0.9	0.4	±4	0.8	-2.4 to 1.6	0.6

SD: standard deviation, SE: standard error of mean, CI: confidence interval

None of the visual function tests showed any significant changes by 6 months. All the changes in visual functions were within the known range of measurement error for each test.

3.2 Ability of the functional tests to distinguish eyes with and without cysts

3.2.1 Introduction: This pilot study is carried out to elicit the ability of functional tests to differentiate the eyes with and without cysts. Recently in a retrospective analysis of ETDRS data, Gangnon and group (Gangnon RE, 2008) assessed ocular factors and systemic non-ocular factors, their regression to baseline visual acuities and found duration of DMO and extent of retinal thickening were correlated to the visual acuities. Here in this pilot study we tried to assess functional tests in eyes with and without cysts to see if there is any trend and whether these tests could be used as screening tools in detecting early progression of DMO.

3.2.2 Material and Methods:

A total of 65 eyes were eligible for this pilot study, of the total 70 eyes screened with diabetic maculopathy for the clinical trial as in chapter 2. All the eyes were grouped into 2 categories based on their OCT findings at baseline: normal OCT (n = 30 eyes) and parafoveal intraretinal cyst (n = 35).

Structural changes on macular cross-sections are best assessed with OCT. On OCT, DMO can also be classified based on the morphological appearance of the fluid accumulation into diffuse, cystoid, or subretinal macular detachment or a combination of these changes, which may coexist with vitreo-macular interface abnormalities.

3.2.2.1 Microperimetry:

Quantification of retinal sensitivity using microperimetry, a fundus related retinal threshold test, allows for exact topographic correlation between fundus details and light sensitivity. The MP1 microperimeter provides a predetermined point-by-point quantification of the retinal threshold of the macula and allows automatic follow-up examination over the same retinal points. Retinal sensitivity is decreased over areas of macular thickening, hard exudates, and haemorrhage in DMO. The microperimeter has been used to evaluate the functional impact of the various subTypes of DMO on OCT and the effects of different treatment modalities for DMO.

In this section we evaluated the ability of microperimetry and other functional tests to distinguish between eyes with no OCT evidence of DMO from those with a central inner retinal cyst due to diabetic maculopathy.

3.2.2.2 Chroma test:

Colour vision deterioration in patients with DMO precedes changes in other clinical measures (Hardy, 1992), is correlated with DR stage (Bresnick, 1985), and is increased in patients with diabetic maculopathy. By measuring visual function, the chroma test could be used to detect and monitor sight-threatening pathology (Wong, 2008) and to identify subjects at risk for severe retinal disease (Ong, 2003). It remains unknown whether colour vision is a sufficiently sensitive means of assessing early DMO, or whether the chroma test is capable of detecting DMO progression.

3.2.3 Statistics: With an expected effect size of 0.2, the sample size required was estimated to be around 393 to obtain a power of 0.8, whilst sample of 65 would give

power of less than 0.25. So our study is expected to be a pilot study to look for the trend of functional test outcome in those eyes where there is a clinically significant amount of macular oedema. The median differences were depicted as Box and Whisker plots.

3.2.4 Results:

Demographic characteristics, mean VA, mean central retinal sensitivity on MP1, and OCT parameters at baseline are shown in Table 3.2.

Table 3.2: Baseline characteristics in eyes with and without fluid on OCT

	Normal morphology	Intraretinal cysts	p-value
Age (years), mean ± SD	55.77 ± 10.89	56.02 ± 11.26	0.92
Gender (male/female)	18/12	25/10	0.61
VA (mean ± SD)	79.93 ± 8.32	77.23 ± 7.61	0.18
Central retinal sensitivity (MP1) (db ± SD)	15.27 ± 3.63	13.34 ± 3.5	0.03
CST (µm ± SD)	238.48 ± 29.08	280.27 ± 52.73	0.0008
Protan threshold	6.64 ± 4.04	7.38 ± 4.43	0.48
Tritan threshold	23.27 ± 22.24	29.27 ± 25.63	0.32
Contrast sensitivity	31.59 ± 5.37	30.85 ± 4.85	0.56

VA, visual acuity; CST, central subfield thickness

Paired sample t-tests of base line characteristics between eyes with cysts and without, using box-whisker plots (Tukey, 1977) are shown in **figure** 3.1-5. In the Box-and-whisker plots, the central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The horizontal line extends

from the minimum to the maximum value, excluding outside and far out values, which are displayed as separate points.

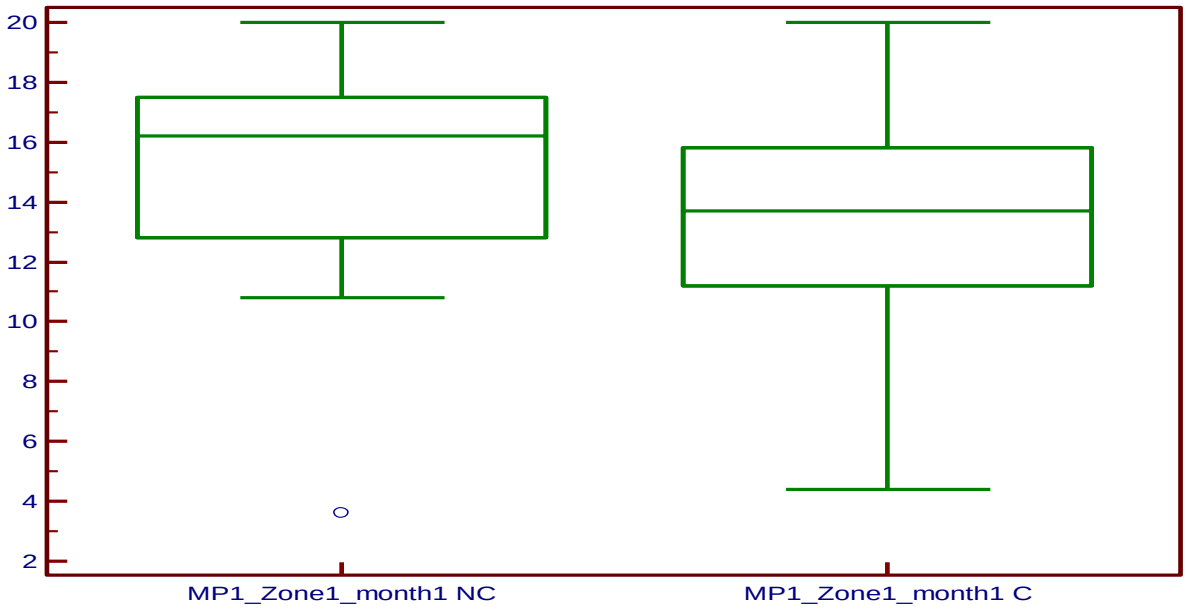

Figure 3.1: Box-Whisker plot for baseline MP1 values. X-axis is retinal sensitivity thresholds in decibels (db). NC: no cysts; C: with cysts

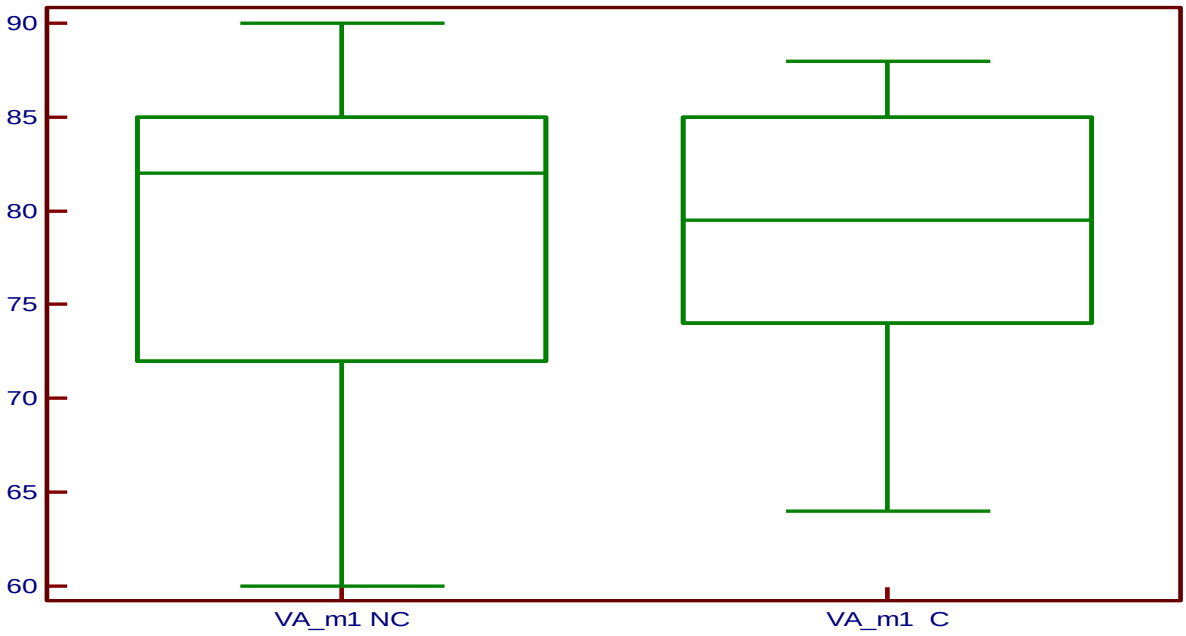

Figure 3.2: Box-Whisker plot for baseline Visual acuity (VA). X-axis is vision in ETDRS letters. NC: no cysts; C: with cysts

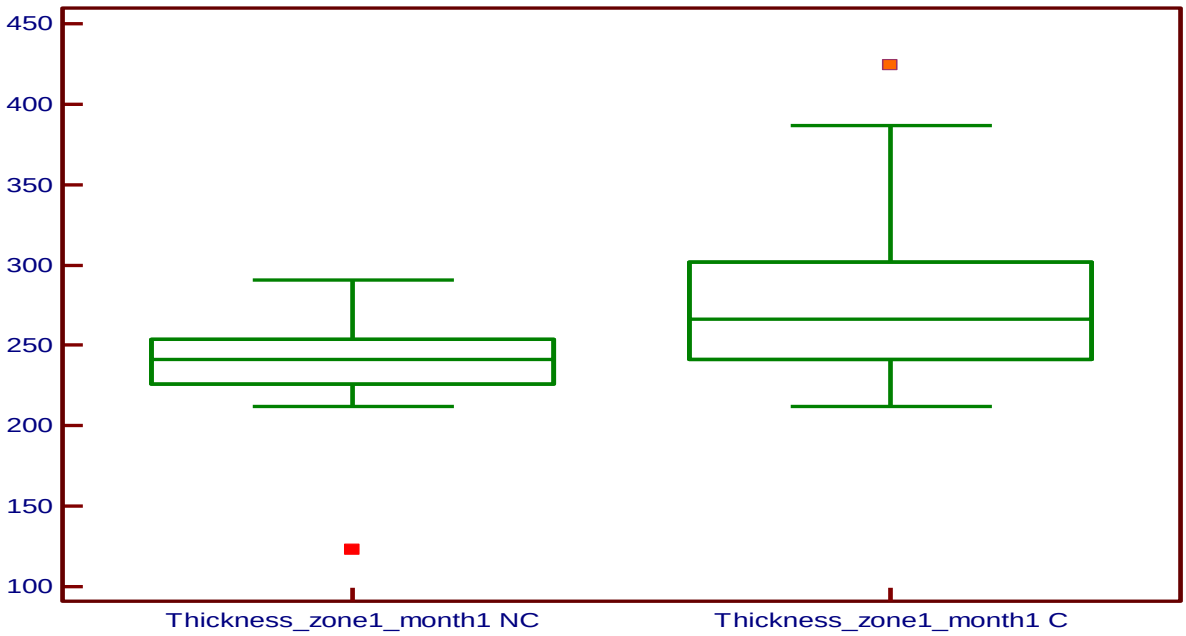

Figure 3.3: Box-Whisker plot for baseline central sub-foveal thickness values. X-axis is retinal thickness in microns. NC: eyes with no cysts; C: eyes with cysts

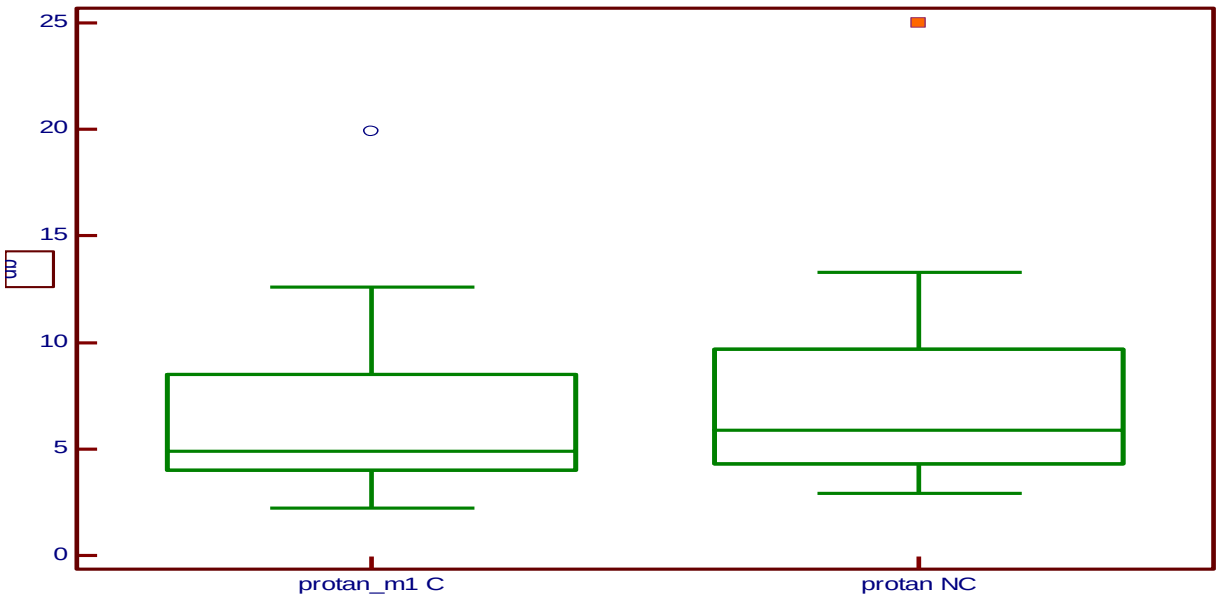

Figure 3.4: Box-Whisker plot for baseline protan threshold values. X-axis is retinal sensitivity thresholds in decibels (db). NC: eyes with no cysts; C: eyes with cysts

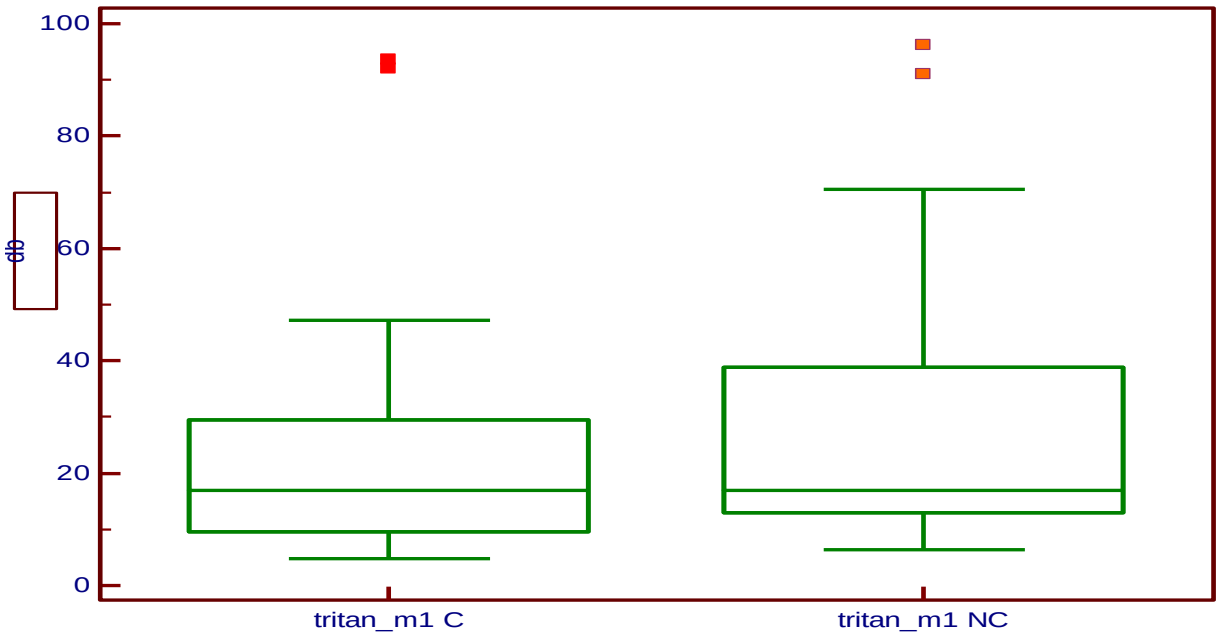

Figure 3.5: Box-Whisker plot for baseline Tritan threshold values. X-axis is retinal sensitivity thresholds in decibels (db). NC: eyes with no cysts; C: eyes with cysts

3.3 Identifying progression of maculopathy

3.3.1 Introduction: An interexaminer consensus was reached to establish progression of all the eligible eyes of the recruited patients as mentioned in section 3.1.2, based on OCT findings. This is a pilot study assessing agreeability between various functional tests. Gangnon and group from ETDRS (Gangnon RE, 2008) also found that in progressive DMO, final visual acuity depends on duration of DMO, location and thickeing of retinal fluid. In this pilot study we assessed whether functional tests could detect minute changes in DMO severity levels and agreeability between various tests, so that these tests could be used in screening early progressive changes.

3.3.2 Material and Methods:

All the eligible eyes from the study mentioned in 3.1.2 were examined by two assessors and a consensus was reached.

3.3.3 Statistics: With an expected effect size of 0.2, the sample size required was estimated to be around 393 to obtain a power of 0.8, whilst a sample of 65 would give power of less than 0.25. So our study is expected to be a pilot study to look for the trend of functional test outcome in those eyes where there is a progression of diabetic macular oedema.

3.3.4 Results:

Of the 65 eyes assessed for structural and functional changes, interexaminer and intraexaminer consensus was as follows: 33 had no structural changes from which to establish progress; 19 had improved on the basis of the OCT appearance of cystic

changes in the retina; and 13 had deteriorated. **Table** 3.3 compares the assessment of detection of progression by various functional tests at 6 months. There was high disagreement among the various structural and functional tests. Only 3 eyes (4.7%) showed total agreement with all of the functional parameters, whereas 12 eyes (20%) had good agreement with all of the parameters excepting any one functional test.

Table 3.3: Progression from baseline to 6 months by various functional tests

	No change	**Improvement**	**Deterioration**	**NA**
Objective OCT assessment	33	19	13	-
Fundus photo assessment	23	18	15	9 (NA)
Chroma colour contrast-Protan	42	13	10	-
Chroma colour contrast-Tritan	30	25	10	-
Contrast vision test	30	18	17	-
ETDRS vision test	36	12	17	-
Mean Microperimetry threshold	40	10	6	9*
Zone 1 Microperimetry threshold	37	13	6	9*
OCT thickness in ETDRS zone 1	31	17	17	-
OCT thickness, initial worst zone	39	21	5	-

NA, not accessible; * Could not complete

Results of the subjective assessment of OCT pictures agreed poorly with the results of the other functional tests. This observation has huge implications on the management of diabetic maculopathy, as it makes it difficult to determine whether maculopathy should be managed solely based on OCT changes, or use functional tests alone. This illustrates the importance of assessing progression by a combination of tests. It also highlights the need to understand the relationship between various structural and functional assessment tools.

3.4 Reliability of the outcome measures

The general conclusion from this analysis is that in diabetic patients with normal thickness macula (defined as central subfield thickness of less than 308 µm on Cirrus OCT), change in any of the psychophysical tests was insignificant over 6 months This study shows that there are minimal changes in visual function over 6 months and variations are more likely to be due to measurement error. If there is an apparent change in test results over a 6 months period, these may be due to real biological changes, instrument or examiner related changes and/or patient related changes. Instrument related factors might include test stimulus variability: size, duration, luminance, background luminance, target size, test strategy, distance of target, intrinsic error in the measurement, lack of eye tracking. Patient related factors include understanding, pre-test training, concentration for lengthier times, fatigue, pupil size, systemic co-morbidities, extent of local damage, underlying retinal pathology, test point locus, under or over correction of the refractive error. In our series of patients all the tests were carried out just before midday. Patients were alerted before about the extended duration for these tests and to avoid hypoglycaemic episodes during their stay for the tests, but still influence of hypoglycaemia on these tests particularly which involve keen observance cannot be ruled out.

Although the majority of our recruited patients are of type II diabetics, a proportion of them were on an Insulin regimen. As part of screening, only OCT analysis was done to rule out diabetic retinopathy related retinal structural changes, but no fundus fluoroscein angiogram (FFA) was carried out to estimate any evidence of ischaemia. This was done purposely to simulate real life scenario diabetic clinic patients, whom could be reviewed periodically without any interventional diagnostic and therapeutic measures. Also worth

noting is that these patients have OCT detectable changes in other eyes, less than CSMO, for the duration of follow-up. So underlying early hypoxic or neuronal functional changes may already be pre-existent in these patients.

Given that none of these visual function changes showed significant variations over 6 months, this suggests that changes in any of these tests can be used as an outcome measure of visual function in a clinical trial in very minimal or no retinopathy when no clinically visible vascular or macular thickness changes are noted. This study does not provide us information on the best outcome measure. However, in the evaluation of candidate visual components for multiple sclerosis clinical trials where the focus is on neuronal changes, ETDRS scores used to measure visual acuity did not change over time (Cutter GR, 1999). On the contrary, contrast sensitivity tested by Pelli-Robson charts in the Optic Neuritis Treatment Trial (ONTT) was shown to be a sensitive measure of afferent visual function, even among patient with Snellen acuities of 20/20 or better (Keltner JL, 2010). Importantly, measures of low-contrast vision are predictive of "real-world" visual tasks such as reading rate, facial recognition, and driving (Leat SJ, 1999).

When the function tests were applied to baseline eyes with and without cysts there was a trend to show deteriorated visual function in eyes with cysts. But when the agreeability was tested to all the function tests based on progression determined by OCT changes, there was very minimal agreeability.

Diabetic maculopathy is a progressive condition that resists even aggressive systemic treatment. Although early management and treatment of diabetic maculopathy are effective, they depend on the early detection of retinal changes. Assessment of progression can be made structurally or functionally; however, functional deterioration

far precedes any structural anatomic changes (Westall, 2005; Jacqueline Lopes de Faria, 2001; Brinchmann-Hansen, 1993; Lieth, 2000.). Increased understanding of the pathogenesis of diabetic maculopathy has shifted the management paradigm towards early detection of changes by functional assessment.

Assessing the retinal status by VA measurements alone does not provide sufficient information to detect early functional changes of the retina. Emerging techniques in OCT have permitted better understanding and quantification of the intricate structural changes that occur during retinopathy. Functional tests allow us to detect damage at earlier stages of the disease process. However, studies have shown varying correlation between structural and functional assessments (Chen, 2011; Kube, 2005; Okada, 2006; Rohrschneider, 2008; Vujosevic, 2006; Trick, 1988; Alkuraya, 1989; Brinchmann Hansen, 1993).

Changes at the macula are responsible for the most common cause of visual impairment in people with diabetes. Visual acuity is the gold standard of assessment of visual function in clinical practice. Visual acuity estimates neuronal function at the fovea, but do not entirely reflect functional vision. Psychophysical tests assess the functional integrity of the entire visual pathway. In diabetic patients impaired night vision and difficulty in detecting the colour contrast and contours are more common complaints even with good visual acuity. Thus, structural changes observed in DMO do not correlate with VA. Functional vision is the impact of vision on the quality of life. This is better assessed by psychophysical tests.

It is imperative that we assess outcomes with reliable psychophysical parameters. Assessments of VA with ETDRS charts (Ferris, 1996), colour vision with special computerized grating methods (Mulak, 2002; Ong, 2003), contrast sensitivity with Pelli-

Robson charts (Elliott, 1990), and retinal sensitivity thresholds with microperimetry (Rohrschneider, 2008) are all well established.

The results of psychophysical tests in patients with diabetes are unpredictable (Brinchmann-Hansen, 1993; Ewing, 1998) and sensitivity varies between tests. For example, contrast sensitivity and colour vision are decreased in diabetics with or without retinopathy (Dosso, 1996; Ewing, 1998). The variability of psychophysical tests, such as VA, in diseased states may be greater than that in normal subjects (Brinchmann-Hansen, 1993; Ang, 2006; Ismail, 1998; Patel, 2008).

The repeatability of normal VA has been established (Raasch, 1998), and ETDRS charts are accepted as the gold standard for VA in clinical trials (Ferris, 1996). Under standard conditions, refraction and VAs are reproducible (Blackhurst, 1989). Previous studies have been conducted to test the repeatability of VA scores on ETDRS charts in normal elderly patients, macular degeneration patients, and patients with other advanced eye diseases (Lovie-Kitchin, 2000; Kiser; Blackhurst; Patel, 2008). Reported estimates of the 95% coefficient of repeatability (CR) of the ETDRS charts range from 3.5 to 8 letters in normal subjects (Elliott, 1988; Raasch, 1998) and from 7.5 to approximately 10 to 15 letters in patients with reduced vision (Camparini, 2001; Kiser, 2005).

Contrast sensitivity (CS) can provide valuable information about visual function related to mobility and orientation in addition to visual acuity assessment (Arden, 1978). Therefore, it is important to establish the intersession repeatability of CS measurements. Studies have tested the repeatability of CS in normal population, using pelli-Robson charts: +/- 2 steps or 0.30 log units (Elliott, 1990), and in diseased eyes: CR of 0.35 log units in registered blind eyes (Kiser, 2005), and CR of 7 letters (0.35 log CS) in eyes with age related macular degeneration (Patel, 2009). Many other studies have also

assessed the repeatability and reproducibility of the Pelli-Robson CS chart in normal patients (Lovie-Kitchin, 2000; Elliott, 1990; Dougherty, 2005; Elliott, 1993) and in those with low vision (Haymes, 2004; Thayaparan, 2007).

Fundus perimetry can be used to quantify macular sensitivity (Midena, 2010; Weingessel, 2009; Rohrschneider, 2008) and its repeatability in normal volunteers is well established: mean macular sensitivity of 19.6+/-0.5 dB in the 20 to 29 years of age group compared with 18.6+/-1.5 dB in the oldest age group of 70 to 75 years (Midena, 2010). The test–retest variability of mean macular and point-wise retinal sensitivity has also been investigated in small samples of patients with early age-related macular degeneration (Weingessel, 2009) and in patients with nonspecific macular disease, co-efficient of repeatability (CR) for mean sensitivity and central macular sensitivity were 1.81, 2.13 respectively (Chen, 2009).

The OCT test is repeatable and reproducible in normal eyes with no macular pathology (Baumann 1998; Garcia-Martin, 2011).

4: DISCUSSION

Current and emerging treatments for diabetic retinopathy

Long-term laser treatment outcome

Laser photocoagulation remains the standard treatment for patients with CSMO. The main objective of laser treatment is to prevent visual loss, rather than to improve vision. Diabetic retinopathy is a leading cause of blindness in developing and developed countries. In current management, disease is diagnosed based on regular clinical examination. Retinopathy progression is determined according to clinical retinopathy changes assessed through OCT scan or FFA. The decision to treat is made based on macular thickening seen on OCT and on FFA, rather than on changes such as vision loss or functional vision deterioration. In most cases, treatment is by laser photocoagulation, a modality that is associated with significant disadvantages and poor clinical effectiveness compared to clinical trial results. Nevertheless, contemporary studies on laser photocoagulation for CSMO indicate that the visual outcomes with macular laser treatment are much better than those obtained with the ETDRS study with approximately 1 in 4 gaining ≥15 ETDRS letters by 3 years (Aiello LP, 2010; DRCRN, 2009). Suggested reasons for this improvement include better glycaemic and blood pressure control and perhaps early detection and prompt treatment of cases compared to a decade ago. However, our study in a clinical setting catering to a multiracial inner city population shows that the long-term results (3–5 years) are inferior to those obtained in clinical trials, with approximately 12% showing improved vision and 26% suffering moderate vision loss at 5 years.

To test the hypothesis that VA may continue to improve in eyes with laser-treated maculopathy (H1), the long-term (5-year) outcome of laser photocoagulation in a real-

life, inner-city population was studied. We were successful in recruiting 100 subjects who were followed-up for 5 years after their initial laser treatment for diabetic maculopathy. The mean annual visual outcomes and systemic parameters were collected retrospectively and compared to outcomes of the laser arm of the DRCRN trial (section 2.1.2). The mean change in VA at 5 years was found to be -5.23, with the 3-year outcome being inferior to the clinical trial results, with more people gaining vision (≥15 letter gain) in the DRCRN group compared to this cohort (26% versus 9%)(section 2.1). Furthermore, 3 times more patients lost vision (>15 letter loss) in the real-life setting of this cohort compared to the clinical trial results of the DRCRN group (27% versus 8%, respectively). These results suggest that laser treatment is only effective at halting—and not reversing—retinal deterioration, which happens with no treatment (ETDRS report 1, 1985). It can be argued that these patients are not trial patients and so not standardized with regard to age; race; sex; diabetic control; other associated systemic conditions, but the outcome suggests a real life scenario, where there is no standardization of the patients. The study has adequate power (sample size calculated for 95% confidence, ± 5%), regular follow-up for 5 years, availability of visual acuities for all the visits, and other systemic functions including blood pressure, blood glucose levels for each visit. Visual acuities were tested by snellen charts according to clinic protocols and were later converted to logmar for data processing. Errors may therefore have occurred whilst assessing vision, because of inter examiner variability, intersession variability, and intrapersonal variability, lack of updated refraction, and to help compensate for these variations, we considered annual mean visual acuities.

This long-term laser outcome study (chapter 2) assessed a number of factors that may determine the poorer outcome. These factors included demographic, ocular and

systemic factors, and issues associated with healthcare provision. Compared to the DRCRN study (baseline data comparing IVTA to laser for DMO), the mean age of the KCH cohort was 4 years younger. The KCH cohort also had more ethnic minority populations than the DRCRN group. The VA examiners were not certified, and VA measurements were recorded in busy clinic settings. Under these circumstances, it is possible that the examiner did not spend enough time encouraging the patient to read as far as possible. As a result, the best-corrected VA (BCVA) may have been underestimated at times. The meanHbA1C of our group was 8.5%, compared to 7.5% in the DRCRN group. HbA1C levels of ≥8 are associated with an increased risk of macular oedema, irrespective of the ethnic group (Chou TH, 2009). In a recent report, it was found that members of the African-Caribbean community are at increased risk of diabetic maculopathy because of genetic predisposition (Gulliford MC, 2010); furthermore, the risk of diabetic maculopathy independent of the ethnic group is significantly higher in subjects registered with family practices with the lowest quartile of HbA1C achievement (Gulliford MC, 2010). The present study results mirror those of the DRCRN group, indicating that the HbA1C levels do not influence the outcomes of macular laser treatment. Thus, decreasing HbA1C levels is more important with regard to the prevention of maculopathy than with regard to maculopathy treatment. This finding suggests that over time, other factors may dominate the course of the disease (Mahdy RA, 2010).

Patients in the KCH cohort also had higher systolic and diastolic BP compared to the DRCRN group. Again, this difference may be explained in part by the differential susceptibility of the African-Caribbean group to high BP. However, unlike the ETDRS study, the DRCRN study reported that baseline systolic BP and mean arterial BP did not

influence VA outcomes. Despite the higher BP in the KCH cohort, univariate analyses did not reveal BP as a predictive factor. As discussed above with regard to HbA1C, epidemiological studies and clinical trials strongly support hypertension as an important modifiable risk factor for DR (Mohamed Q, 2007). In the UKPDS study, tight blood pressure control reduced the risk of retinopathy progression by about one-third, visual loss by one-half and the need for laser treatment by one-third in patients with type II diabetes. Similarly, the EUCLID study (Chaturvedi N, 1998), DIRECT study (Sjolie AK, 2008) and RASS study (Klein R, 2006) all showed positive outcomes for antihypertensives on DMO risk. However, these are risk reduction strategies for the development and progression of DR. Although both HbA1C and BP must be optimally controlled to decrease the rate of incident DR and DMO, they do not appear to influence laser treatment outcomes, as shown in the current study and based on the analysis of the DRCRN group (Aiello LP, 2010).

Other factors that could have influenced the outcome were certain inclusion and exclusion criteria. DRCRN has completely excluded patients with chronic renal failure whilst KCH group had 15 patients with chronic renal failure requiring dialysis treatment. Also 13% of DRCRN cohort had additional treatments along with laser, whilst KCH cohort had none excepting laser treatment. Similar ceiling and floor effects were seen because of the amount of improvement that can occur when acuity is only mildly reduced and the amount of worsening that can occur when visual acuity is poor.

Due to the large number of variables evaluated, we only considered associations with a P-value < 0.01 to be significant. Variables that met these criteria included: insulin users, number of laser applications, number of missed clinic appointments, baseline VA, and BMI ≥ 25. The possible explanation could be that patients who are already on insulin

have advanced macular oedema with poor visual acuity on presentation and so less influenced by ceiling effect. Similarly those who had more number of laser sessions and higher BMI have had poorer baseline visual acuities to present with and the improvement in number of letters with laser treatment is unaffected by ceiling and probably influenced by floor effects in minimising the deterioration. Nevertheless, a few of the variables met a $P < 0.05$ value threshold in multivariate analyses; they were the univariants (i.e., being on insulin medication and baseline VA). Similar to the analyses of the DRCRN group (Aiello LP, 2010), we found that visual improvement was better in eyes with poorer baseline VA (< 55 ETDRS letters). These types of ceiling and floor effects have been reported for treatment outcomes associated with both diabetic maculopathy and age-related macular degeneration (Aiello LP, 2010; Boyer DS, 2007). The duration of oedema may be an important determinant of final visual outcomes, but this factor was not analysed directly in the current study (Gangnon RE, 2012). Nevertheless, the poorer results in year 3-5 may serve as a surrogate marker of chronicity of disease.

Despite the fact that all our patients were treatment naive at baseline, the mean number of laser applications was only 2.7 at 5 years compared to 2.9 at 3 years in the DRCRN group. Although it did not reach a significant level in the multivariate model, the number of laser applications is an important factor that may have influenced our outcomes. The high threshold among retinal specialists to perform more lasers when 2–3 attempts have not shown a positive response should change, based on recent data reported by the DRCRN indicating that the probability of improvement of eyes treated previously with laser ≥ 3 times had a similar chance of VA improvement as eyes that had not had prior laser treatment (DRCRN, 2008). Taken together, these findings

suggest that it is useful to proceed with further laser treatment if there is sufficient space to apply more burns. Response to laser treatment is slow, and persistent oedema after 1–2 laser treatments should not deter physicians from repeating the laser treatment.

Another significant problem in the real-life setting of urban populations is lack of awareness of diabetic retinopathy and its associated complications. Twenty-two percent of the patients in a recent study of urban populations failed to attend screening appointments (Gulliford MC, 2010), with the highest nonattendance reported among 18–34 year olds. In the current study, approximately 50% of the subjects were not followed-up regularly, and 16% were lost to follow-up. Therefore, these results may be worse than reported if the outcomes for the lost patients were known. The current screening and treatment guidelines ensure that patients with sight-threatening disease are promptly referred and treated. However, the major challenge of providing timely monitoring and treatment appointments for these patients remains unaddressed.

The strength of the current study is that it included the largest number of patients with DMO who had macular laser treatments in real-life settings with long-term follow-up, thereby allowing the results to be compared with outcomes from contemporary clinical trial results. However, a limitation of this study is its retrospective nature. Despite the fact that consecutive patients with 5-year follow-up were recruited, approximately 50% of the patients did not complete the 5-year follow-up or did not have at least one annual follow-up visit during this time period. Therefore, it can only be postulated that the results may be inferior to the present data if all patients would have been followed. Finally, this study did not differentiate between focal and diffuse macular oedema, as angiograms were not available in all cases.

In summary, this study of long-term laser treatment outcome shows that retinal specialists should contemplate further laser treatments in patients with persistent oedema despite potential initial non-responsiveness to laser treatment. Rigorous measures should be initiated to ensure timely follow-up to avoid non-attendance and resultant loss of vision of these high- risk individuals. The theory behind the way laser works still remains elusive. Should the laser photocoagulation, which is permanent destruction of retinal pigment epithelium and the overlying photoreceptors, improve retinal oxygenation, the results should sustain in long term. But the results are contrary to this, and the mechanism of action of laser photocoagulation in diabetic maculopathy is inconclusive. So to evaluate if there is any effect of increased oxygenation, another trial was conducted where improved oxygenation of the retina was achieved by inhibiting the rod activity.

Given that hypoxia contributes to the pathogenesis and aggravation of DR and DMO, and because photoreceptors utilize oxygen maximally during dark adaptation, the next part of the research aimed to test the hypothesis that decreasing the oxygen consumption by the photoreceptors via reducing dark adaptation may have a positive impact on diabetic maculopathy (H2). A phase II clinical trial was conducted to test whether inhibiting rod dark adaptation could reduce the oxygen load on the retina and halt progression of retinopathy (section 2.2).

Outcome of the light mask trial:

Thirty-five patients with treatment–naive DMO were assessed during and after a 6-month period of wearing masks that illuminated the lid of one closed eye during their night time sleep. Patients included those who had DMO that was non–sight threatening

or too close to the fovea to laser. No adverse effects were encountered; and none reported any sleeping or visual disturbances. Further tests are required to see if there is any additional effect of heat generated, if any, as this could possibly cause production of heat shock proteins, which might have a protective effect against some types of cell injury. Morphologic changes in the illuminated eyes were monitored by OCT. Improvements were seen with intraretinal cysts, exudates, and retinal thickening, most of which disappeared or were substantially reduced with mask treatment. All these changes were noted in the first three months, and continued during the second 3 months. Tests of visual function in the study eyes improved (both achromatic and colour contrast sensitivity), whereas those in fellow eyes did not improve or worsened. Mean retinal sensitivity was improved and a slight increase in VA was observed in the study eyes. Because light therapy is inexpensive compared to the currently available interventions, and does not require medical supervision, its early adoption could possibly prevent or decrease progression of DMO. Further random control trials are required to consolidate the results.

The probability that the improvement in the trial eyes in this study occurred by chance, or that such changes could be accounted for by regression to the mean, is extremely small. One possible explanation of this result could be that the OCT appearance fluctuated in time and, by chance, the first observation caught the condition at its worst, so that the later measurements would be nearer to the average and the apparent improvement would be fallacious (regression to the mean). But the probability that the final measurement would be larger than the initial is exactly the same as the other condition, so the binomial probability would indicate the likelihood that regression to

the mean occurred. In general, diabetics with DMO get worse with time; therefore, the probability that the result could be obtained by chance is much lower.

It is the common experience that untreated eyes with DMO worsen. In addition, in all the measures we used, retinal function improved in trial eyes. For most tests, this improvement was statistically significant. The sole exception was the VA, for which only a small increase in the number of ETDRS letters read was noted. However, in our patients the VA was normal or nearly normal prior to the trial; thus, the changes are subject to the "ceiling effect". By contrast, there was deterioration of these other measures of function in the untreated eyes. Most of the improvement occurred within the first 3 months of the trial, although continued improvement was also observed in the latter half of the short trial.

Trial design and failings

One difficulty was the high failure rate of the masks, such that some patients did not have continuous periods of illumination at night. We had no means of determining whether the light-emitting diodes were always positioned in front of the pupil, or if they could become displaced during the night. These failings in the mask are considered to reduce the efficiency of the treatment and increase the significance of any improvement detected.

Comparison to Present-day treatments

Although a series of treatments of DR are available, they are all invasive (except diet control) and some (e.g., pan-retinal photocoagulation) inevitably reduce visual performance (Massin P, 2010). In addition, considerable skill is required to carry out repeated intravitreal injections or laser treatments with safety. Delivering light therapy is a cheaper and non-invasive option to treat early diabetic maculopathy. Our trial

patients accepted this method of treatment very well and none reported any side effects. Although it is conceivable that present-day treatments might be given in areas with a shortage of basic medical facilities, the number of patients with diabetic eye disease is so great that employing the forms of treatment readily available puts great strain on most state-funded health services. In addition, none of the treatments is inexpensive.

Comparison to other trials

Most previous trials of treatment have been designed to test the efficacy of invasive methods; therefore, treatment has been given to patients with quite advanced disease. The patients in this trial do not meet ETDRS criteria for focal laser treatment. Therefore, direct comparison with other trials is difficult and our results cannot be directly used to estimate the value of the treatment for patients with more advanced disease.

Implications of results for the causes of diabetic retinopathy

The total power consumption of the electronics of masks was <2 mW. If this power were converted into photons with 100% efficiency (which is impossible) and all of those photons were distributed evenly on the retina, then the power would be ≈ 1 mW mm^{2}. Because of the attenuation of the light by the lids and the media (Harding S, 2003; Robinson J, 1991), the actual maximal power incident on the retina must be approximately $1/100^{th}$ of this figure. Such a low light level cannot conceivably cause damage to the retinal structures, significant heating, or affect the mitochondrial function. However, alteration in the light/dark cycle can alter circadian rhythms, via activation of ganglion cells that contain melanopsin (Berson DM, 2002), and such alterations are associated with various disturbances, including an increase in the incidence of various cancers (Stevens RG, 2009). The action spectrum for modification

of the production of melatonin has been determined, and the retinal illumination of 480 nm light required to cause a 50% reduction in the night-time increase of melatonin is 25 uW cm^{2} (or 10^{14} photons cm^{2} s) (Brainard GC, 2001; Thapan K, 2001). Therefore, the trans-lid illumination caused by the masks would produce minimal changes in melatonin, and the effects on DMO must be mediated via absorptions in photoreceptors and activation of the transduction mechanism.

The only known way for such weak light to reverse changes in early DMO is via the hypothesis already advanced (Arden GB, 2005): namely, that during dark adaptation, rod metabolic activity increases and the resulting hypoxia stimulates production of cytokines that cause retinal damage. Thus, the trial must be considered as a proof of principle. It naturally follows that many of the damaging biochemical changes in DMO, which have been analysed in detail, must be secondary to the insult caused by anoxia.

Study limitations

No adverse effects were encountered, but I have not investigated whether continuous light interrupts the synchronous shedding of rod tips that occurs each dawn (Schremser JL, 1995). Each RPE cell in the region of maximum rod density has to engulf approximately 20 mm^{3} of solid material each day, which is roughly its own cytosol volume; this material is presented in the course of a few minutes. If this shedding process did not occur, then the ability of RPE cells to phagocytise and digest rod discs might actually improve. Any adverse effect of low intensity continuous illumination is most unlikely: there must be a very large number of night workers who sleep in the day under mesopic conditions, and such employment has not been associated with any retinal damage. In rodents, which are very susceptible to light damage, increasing the average 24 h illumination within tolerable limits only results in a shortening of the rod

outer limb, which is auto regulatory, and results in the cell absorbing a constant 14,000 photons/rod/s (Schremser JL, 1995; Williams TP, 1998, 1999). For this study, it was calculated that the illumination of the light masks provided only 50-500 photons absorbed/rod/s.

Less than 50 patients have undergone trials with light-masks in this phase II trial, and much larger trials must be undertaken with longer periods of observation. The patients all had early stages of DMO, and it is not known whether light would be effective at advanced stages; therefore, additional trials, both of pre-proliferative DR and advanced DMO, would be desirable. Investigations of different populations (e.g., adolescents or people of different ethnic composition) to determine if they react in the same manner would be of interest.

Significance for diabetic retinopathy

Providing light during sleep is inexpensive, and methods of obtaining it are readily available in places where there is a constant electricity supply. However, the use of light-masks similar to those we have made has certain advantages: the intensity of light is known and is not subject to the patient covering the eyes. Furthermore, the cost of running the mask compares favourably with the cost of electrical room illumination. Provision of light at night, from whatever source, does not require tertiary medical supervision, or any supervision at all. It would be possible to make masks with mechanical chargers or solar-powered chargers in the absence of a reliable electrical supply. Therefore, even if the method only functions to reverse the earliest retinal diabetic changes, it might be widely adopted by people with diabetes of duration longer than a critical period, perhaps 10 years; this would reduce the global epidemic of DR and its complications to easily manageable proportions.

Current and emerging diagnostic approaches for diabetic neuropathy

Short term fluctuations in functional tests

Assessment of the reliability of various functional tests is crucial for patients with early diabetic macular oedema. Their systemic and ocular management could be influenced by the outcome of these tests. Because retinal neuropathic changes are thought to precede structural changes, it is imperative to assess the progress. Any psychophysical test is associated with an inherent measurement error.

Factors that could affect reliability of the outcomes can be categorized as instrument-, examiner-, or patient-related. Instrument-related factors include test stimulus variability, size, duration, luminance, background luminance, target size, test strategy, distance of target, intrinsic error in the measurement, and lack of eye tracking. Patient-related factors include understanding, pre test training, concentration for lengthier times, fatigue, pupil size, co-morbidities, extent of local damage, underlying retinal pathology, test point locus, and under- or overcorrection of the refractive error (Heijl, 1989; Werner, 1990).

In our series of patients, all tests were carried out just before midday. Patients were alerted before about the extended duration for these tests. They were told to avoid hypoglycaemic episodes during their stay for the tests, but the influence of hypoglycaemia on these tests cannot be ruled out. Although most of our recruited patients were Type II diabetics, some were on an insulin regimen. As part of the screening, only OCT analysis was done to rule out DR-related retinal structural changes; FFA was not performed to estimate evidence of ischaemia. This choice was made to simulate real-life scenario of diabetic patients in the clinic, whom we would review periodically without any interventional diagnostic or therapeutic measures. The

patients had OCT-detectable changes in the other eye (less than CSMO) for the duration of follow-up. Underlying early ischaemic and neuropathic changes cannot be ruled out that could influence the outcome of these psychophysical tests.

Assessing the functional test outcomes in these eyes gives us better understanding of the variability expected in these compromised eyes, even if they are not showing any clinical signs of ongoing pathology. The functional outcome of these tests should give realistic expectations of the eyes assessed in DR clinics. Although no deterioration was noted on OCT in some of the eyes observed over a period of 6 months, neurologic deterioration cannot be ruled out. Various sources of variability were found, including those related to patient age, diabetic status, and ability to concentrate, as well as to instrumentation stimulus intensity, size, distance, and duration of tests. Although every measure was taken to ensure that the fatigability did not influence the outcome, nevertheless, errors could have crept into our results.

The CR has been established in normal individuals, including early age related macular degeneration with no choroidal neovascular membrane (Patel, 2009; Patel, 2008) and other nonspecific macular conditions (Chen, 2009). We conclude that these tests haven't shown much progression in short term follow up periods, but taken with caution, because of the underlying ischaemic and neuropathic changes that happen before structural changes are evident clinically.

The retinal thresholds by microperimetry in the sub-foveal region seemed to fluctuate more than the mean or peripheral sensitivity, due to the central subfoveal zone that is much more influenced by early macular ischaemic changes than the para- and peri-foveal zones. This situation led us to assess only those eyes with no change in OCT-based retinal thickness specific to the central subfoveal zone.

Despite these limitations, the implications are clear: no one test can be used to assess progression completely, and there is poor agreement between the tests in assessing progression. Therefore, combinations of tests are required to assess DMO progression and the influence of various new therapeutic interventions on macular oedema.

Ability to differentiate eyes with cysts and without at baseline

New diagnostic modalities are required to assess early functional visual loss and allow for early intervention. Vision assessment is a gross functional measure. With the onset of new therapeutic modalities in treating DMO, better assessment tools are required to improve functional outcome.

Given that neuronal changes precede vascular changes, this study aimed to test the hypothesis that tests of visual function may be a better screening option than grades of DR. The objectives were to examine the relationship between structural changes in the macula and changes in visual function, in 70 eyes of 35 diabetic patients with treatment-naïve diabetic maculopathy. The results of functional assessments, including VA, colour contrast sensitivity, and MP1, did not correlate to structural changes at the macula with OCT and colour fundus photographs. These findings may suggest that: 1) neuronal changes, as denoted by visual function tests, are independent of the visible structural changes on OCT of the macula denoted by intra retinal cysts; 2) other structural parameters on OCT that better estimate visual function should be explored; 3) OCT may not be sensitive enough to detect ultra structural changes within the retinal layers and so there is more scope for new technology to look more in depth into the structural changes; 4) a combination of tests could be considered when assessing these patients.

Detection of retinal macula progression by functional tests and OCT

Various psychophysical tests could be used to identify early functional changes in the diabetic retina. For instance, the chroma test (Wong, 2008) can be used to determine colour vision thresholds, CS can provide valuable information about visual function related to mobility and orientation, in addition to VA assessment (Arden, 1978), and fundus perimetry can be used to quantify macular sensitivity (Rohrschneider, 2008). All of the functional tests used in this pilot study did show progression to variable extent in eyes with definite OCT-based deterioration.

There was high disagreement among the various structural and functional tests. Only 3 eyes (4.7%) showed total agreement with all of the functional parameters, whereas 12 eyes (20%) had good agreement with all of the parameters excepting any one functional test.

Results of the subjective assessment of OCT pictures agreed poorly with the results of the other functional tests. This observation has huge implications on the management of diabetic maculopathy, as it makes it difficult to determine whether maculopathy should be managed solely based on OCT changes, or use functional tests alone. This illustrates the importance of assessing progression by a combination of tests. It also highlights the need to understand the relationship between various structural and functional assessment tools.

Structure-function disagreement

As yet documentation of disease progression is one of the most challenging aspects in the management of diabetic maculopathy. Diabetes affects the neuronal functions of the retina and, hence, the psychophysical aspects of vision. Functional changes begin to

occur earlier than structural changes. With emerging techniques in assessing the structural morphology of the retina, we are able to better understand and quantify the intricate structural relations; meanwhile, functional tests assessing retinal sensitivity aid in the detection of very early retinal damage.

Although OCT is considered to be the gold standard for assessing structural changes of the retina, OCT results do not always correlate with functional changes. Meanwhile, VA is considered to be the gold standard for assessing disease progression, but VA results do not correlate well with progressive structural changes. In this study, we tried to assess whether there is significant correlation between results by MP1 and OCT, and whether MP1 could better detect progressive changes than VA.

Assessing the visual function by OCT, MP1, and VA

Assessing retinal status only by VA measurements does not provide sufficient information to detect early functional changes of the retina. Macular function is not fully characterised by VA, and testing this functional aspect alone ignores central and para-central scotomas that could influence functional vision. Microperimetry mainly assesses the photoreceptor function, whereas OCT only assesses structural integrity within the layers in DMO. Whereas light sensitivity is reduced in areas of macular oedema, various studies have shown no correlation between the amount of oedema and visual function (Rohrschneider K, 2008); no correlation between macular oedema and light sensitivity threshold values (Kube T, 2005; Okada K, 2006; Rohrschneider K, 2008; Vujosevic S, 2008; Trick GL, 1998, Alkuraya H, 1989; Brinchmann Hansen O, 1993); and differing correlations between OCT thickness, MP1, and colour and contrast sensitivities in assessing DR (Okada K, 2006).

This study shows that not all OCT-related changes cause functional changes. Although previous studies have shown a strong correlation between CSMO changes, VA, and retinal sensitivity, no significant relation could be found while assessing progressive macular oedema. OCT-related cystic changes may fluctuate (Coefficient of repeatability (CR): 7.67μ) without actual functional deterioration, leading to high disagreement between structural and functional tests in assessing progression. This disagreement has huge implications on diabetic maculopathy management. Although the poor agreement could be partly due to measurement errors, there are undoubtedly other aspects, because the tests do not all measure the same retinal functions. Moreover, the association between structure and function of the retina would varyingly influence the outcome of these tests. These findings may suggest that neuronal changes, as denoted by visual function tests, are independent of the visible structural changes at the macula and should be used in conjunction with OCT parameters. This finding illustrates the importance of assessing progression by a combination of various tests, and of understanding the relationship between various structural and functional assessment tools.

Although the tests were carried out under strict protocols, a few limitations should be noted, including short duration of the assessment period, lack of control over the glycaemic status of patients during the tests, and lack of control over the intensity and duration of the tests, which puts extra pressure on the diabetic patients. Although progressive cystic changes in the eyes were compared to eyes with no changes, neurologic deterioration over the 6-month period cannot be ruled out; this effect could have influenced the functional parameters.

Problems with MP-1

MP1 documented individual areas where function was altered and correlated well with OCT changes; however, OCT changes did not statistically influence the mean MP1 values or central MP1 thresholds. Therefore, although the point-based threshold values are good to follow, use of zonal summative thresholds similar to the zonal thickness given on OCT scans should be considered.

This study shows that the measurement of macular sensitivity by microperimetry is a better tool for evaluating visual function than VA in eyes with early diabetic maculopathy. However, such measurements do not correlate with the CST, because the pathology could be anywhere in the macula in the early stages. Moreover, the mean and central MP1 thresholds are not an accurate tool to assess disease progression, unless the MP1 thresholds are subcategorised into subzone regions. This result is similar to the case with OCT, in which CST does not correlate well with other psychophysical functions, including VA. For microperimetry to be used as a screening tool to assess progression, the British Diabetic Association has indicated that the sensitivity and specificity must be as high as 80% and 95%, respectively.

Although an exact correlation between fundus disease and functional defects can be achieved by integration of fundus imaging with computerized thresholds, it is not possible to follow-up the patients with zonal values. Therefore, obtaining central or mean values to correlate is of utmost importance; in this study, microperimetry failed in this regard. The current practice is to assess the functional impact of DMO by quantifying the best-corrected VA (BCVA), even if this parameter is just one aspect of macular function. However, MP1 has other drawbacks, including a sharp learning curve, time consumption, reliability, and cost effectiveness.

Chroma outcome

Although VA is considered to be the gold standard for assessing progression of DR, it does not correlate well with progressive structural changes. In this study, we assessed whether tritan colour thresholds on the chroma test correlated better than VA with OCT subfoveal thickness. Seventy eyes with diabetic maculopathy were tested, including eyes with or without parafoveal intraretinal cysts according to OCT. No significant relationship was found between the 2 functional parameters and the structure-function correlation, in terms of the ability to differentiate OCT findings. Though both chroma tests and visual acuities failed to differentiate eyes with and without cysts at baseline, tritan thresholds were better at detecting progressive changes than VA, indicating a potential use for the chroma test in this application. But again colour vision and contrast visions are gross functional assessments like visual acuity, and doesn't provide details of localised changes as seen on microperimetry. Protan and in particular Tritan colour vision has been shown to be influenced by diabetic retinopathy (Treager, 1993). The most likely mechanism for this effect is thought to be reduced oxygen saturation levels in outer layers of retina. (Dean, 1997).

The chroma test machine is a prototype and errors could have crept in which weremachine related; fatigability; and difficulty in controlling testing conditions. We tried to minimise these by doing the tests with the same observer, same time of the day each visit, since all these patients are diabetics and fluctuation in glucose levels anticipated. Though the colour vision thresholds weren't as sensitive as OCT changes, they were on par with visual acuities and better in detecting progressive changes. More refinement of the procedure is required. Future longitudinal studies with a larger sample population are needed to confirm these findings.

CHAPTER 5: CONCLUSION

Current management of diabetic macular oedema involves diagnosis according to clinical structural changes (which appear much later than diabetes-associated functional vision changes), treatment decision according to macular thickening seen on OCT and on FFA (which are time-consuming and labour-intensive), and treatment by laser photocoagulation (which is associated with collateral damage and very poor clinical effectiveness in terms of its ability to reverse damage). With the development of new therapeutics in the management of diabetes, including VEGF inhibitors, newer antidiabetic agents that only indirectly affect insulin secretion, as well as incretin receptor agonists (i.e., exenatide) and enhancers (i.e., sitagliptin) that increase the activity of GLP-1, prevention of functional vision loss in DMO has become an achievable goal. Detection of early functional changes will require better diagnostic tools than are currently available. Continued research is needed into improved diagnostic and treatment modalities to halt the progression and improve the outcome of management of DMO.

Possible future directions to this research are many:

- Our findings that laser photocoagulation is far less effective in clinical practice than clinical trials and should be verified in long-term studies among larger populations. Steps should be taken to identify possible reasons for the discrepancy between trial and real-life findings. The various confounding factors could be age, race, ethnicity, diabetic control, blood pressure control, threshold for laser treatment, earlier detection and intervention, compliance of patients.

- Very optimistic results were obtained with a phase II clinical trial of rod dark adaptation by illuminated night-time masks. Given the cost-effective and non invasive qualities of this therapy, the results could have far-reaching applications for DMO management. However, this study was hampered in part by a high failure rate of the masks (approximately 30%). Additional trials, with improved electronics, larger sample size, in patients with varying degrees of severity of retinopathy, should be performed.
- The results on the study of function-structure correlation for various visual functional assessment methods indicate that currently available diagnostic tools are inadequate for the early detection of functional changes related to DMO. Although these results may indicate that neuronal changes on visual function tests are independent of the visible structural changes on OCT, additional studies are needed to verify this claim. Moreover, additional research should be done into novel methods for detecting visual changes at the very early stages of retinopathy, when functional visual defects occur.

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7. APPENDICES

A) Instructions for our collaborators -How to use the light mask.

When we give you the light mask, we will make the adjustments, which will bring the light onto your closed eyelid. You will be able to see the green light through your lid. The light should seem to be over a wide area, and roughly "straight ahead".

Putting the mask on

When you put the mask on yourself, you will have to learn to position it correctly. This may be a bit fiddly for a day or two. The most important thing is it should not be too tight, because then it will be uncomfortable to wear. If your hat size is 7 ¼ or more, the elastic bands should be where the top of your neck joins your head, not over the bump at the back of your head. If your head is smaller then the bands should go higher. We could only make one size for this research and it has to do for everybody!

Hold the mask a little way away from your face. Open your eyes and position the little lighted disc which so it is in front of your eye. Then close your eyelids and let the disc contact the lid. You should see the dim green light through your lids. If you cannot the disc is not in position. Move the disc and try again. As we said, the mask may not be a good fit: You can move the mask a little to make it more comfortable, and also, to make the light more central: try moving it from side to side, and up and down. You should be able to see the green patch when the room lights are on. Do not try and make the light as bright as possible by pressing the mask against your eye- that is not needed.

What to do when you take the mask off

In the morning, when you take the mask off, place it face-side up on the charging plate. Then move it around till the light goes off. Then turn on the switch on the side of the box, so the charger is on: a little green light will appear on the top of the box. In the evening you can turn the charger off. You should charge the mask every day, but if you forget, the battery will last several nights. If the battery in the mask goes completely flat, it may be difficult to get it to recharge properly.

WHEN YOU COME UP TO THE HOSPITAL REMEMBER TO BRING YOUR MASK!!!!

If you have any trouble you can phone 02070408863 OR 0207 3599080….

B) GP LETTER

Project number [08/H0808/198]

Dear Dr. …..

Your patient ……..

who is currently attending our Diabetic/ Eye clinic, has been approached by us, as part of a research project we are conducting at Kings College Hospital NHS Trust We are trying to obtain evidence to test the hypothesis that sleeping in complete darkness is a predisposing cause for diabetic retinopathy, and we are asking patients to sleep in a " light mask" for 6 months. The mask is similar to ones on sale, but ours will illuminate only one retina (through the closed eyelid) during the hours of sleep. We believe that after 6 months we will be able to show a change in the progression of diabetic retinopathy in the patients' illuminated eyes relative to the unilluminated. The light intensity is very modest, and cannot cause harm. The mask itself is inherently safe to wear, and well tolerated in a small preliminary trial. If any participant found it uncomfortable or difficult to sleep in, we would end the individual's participation in the trial. The trial is being conducted " blind" and we therefore cannot inform your patients or you of their individual results during the trial, which l will last 1 year. At the end of the trial, we will know if our work supports the hypothesis, and can give you more information.

Yours sincerely

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Project number [08/H0808/198]

C) CONSENT FORM

Title of Project: Dark adaptation and Diabetic Retinopathy
Names of Researchers:
Miss S. Sivaprasad Professor G. Arden
1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions.
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.
3. I understand that sections of any of my medical notes may be looked at by responsible individuals from [City university] or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. I explicitly agree that the information of the research project and my name, date of birth, and hospital number may be recorded on a computer, so that in the future comparison can be made with my clinical records
4: I agree that you can tell my GP, I am participating in the study
5. I agree to take part in the above study.

Name of Patient or subject Date Signature

Name of Person taking consent Date Signature

Project number [08/H0808/198]

D) PARTICIPANT INFORMATION LEAFLET

1. **Study title:** Diabetic Retinopathy and Dark Adaptation
2. **Invitation paragraph**

You are being invited to take part in a research study. Before you decide to join it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

3. **What is the purpose of the study?**

Diabetes is a very common condition, and although it is trea**Table**, there may be serious complications. After many years, the eye may suffer, particularly the light sensitive retina. This is called Diabetic retinopathy (DR). We believe we know the cause of this complication and are trying to test our ideas by a clinical trial. If we are right, then it would be a considerable step in preventing this serious condition.

4. **Why have I been chosen?**

You have been chosen because you have diabetes, and there are early signs in the back of your eye that some changes associated with DR have begun and that both eyes are similarly affected. Also your general health is good.

5. **Do I have to take part?**

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

6. **What will happen to me if I take part?**

If you are interested in taking part in this project, we want you to go to sleep every night, wearing a "light mask" (which we will show you), that will shine light onto your eyelid while you are asleep. We think that this light will improve your retina relative to the other (which will remain in darkness). We will only treat one eye, chosen at random, because that will give us a rapid way of assessing whether we are right or wrong. We do not want to treat only "worse eyes" because this would influence our result.

7: **What do I have to do?**

We will fit you with a light mask, and ask you to take it home and try it out. We ask you to return after a week. If you feel you can use it without discomfort and loss of sleep we will then ask you to volunteer for our trial, and ask you to sign a consent form. We will give you detailed eye examinations of the sort you have already had after 3 and 6 months. This means you will have to come to the Hospital once or twice more often than if you were not in the trial. We will contact you at home in-between, to check on how you are getting on. We are not asking you to make any other change whatsoever in your life. If you are already having treatment of any sort it will continue.

8: What is the procedure that is being tested?

In complete darkness, the retina becomes more sensitive, and it does this by using much more oxygen than in light. We believe this extra oxygen cannot be completely supplied in people with diabetes, and this is the cause of the DR. You only dark-adapt when you sleep in the dark. We are trying to prevent this in one eye. At the end of the trial, of course, if we are right we could treat both, but the comparison between your right and left eyes is important.

9: What are the possible disadvantages and risks of taking part?

We cannot think of any disadvantage except that you may not be able to sleep undisturbed when wearing our mask. If so, we want you to stop and return it. The mask is designed not to cause any damage or irritation.

10. **What are the possible benefits of taking part?**

It is not likely that you will get any personal benefit from taking part in our trial , but if we are right, you will be among the first to benefit from the later developments.

11. **What happens when the research study stops?**

We will analyze all the data, and publish it. If our ideas are correct they will be taken up everywhere in the world. We will keep you informed of the results when the trial is over. There are various ways in which you could continue to sleep in light, if you wanted to, and we could advise you about them and help you.

12. **What if something goes wrong?**

We believe that our test cannot possibly do you or anyone any harm. If you have complaints, you should address them to the investigators, (names addresses and telephone numbers given below). If you want to complain about the way the study has been conducted you can use the normal complaints mechanism available to anyone receiving care in the National Health Service. Your right to use this service is not compromised in any way because you have taken part in a clinical research study. You can additionally complain to a Research Administrator at City University – for names see below. These people will deal with any complaint whether it is related to the trial itself, or any other experience you have while attending the outpatients department. We are legally bound to tell you the following:

> 'If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.'

13. **Will my taking part in this study be kept confidential?**

For purposes of analyzing the data, some information about the state of your eyes will have to be recorded, and of course we will have to have details so we can have access to records in the future. Therefore we will need to record your name, date of birth and hospital number, and also we want to contact you at home so we would like your address and telephone number We plan to keep this data on a computer at City University. London. The computer will be password protected, and kept secure. The data will also be encrypted. Data such as this is always transferred to discs, in case the computer breaks down. Such discs will also be kept

securely. The data-analysis will remove any piece of information that could be used to identify you and all the data will be kept strictly confidential. If you agree we will write to your GP about our work and explain you have volunteered. You might also like to consult him before agreeing.

14. **What will happen to the results of the research study?**

When the research has been analyzed we expect to publish in major journals related to eye disease and to diabetes. We cannot tell you exactly when such a publication would occur or where. If it appears that the results might be of general use, you are likely to hear about it from the press, and we would produce a leaflet to be distributed in this clinic.

15. **Who is organizing and funding the research?**

This research has not been paid for in whole or part by any public body or commercial firm.

16. **Who has reviewed the study?** The Research & Ethics and the #research and Development Committee of the King's College Hospital NHS Trust

17: **Contact for Further Information** Professor G.B. Arden, City University. Northampton Square EC1 V 0HB Tel 0207 040 8863 or out of working hours 0207 359 9080 g.arden@city.ac.uk

Dr. P Bunting, Research Administrator, School of Community and Health Sciences City University Northampton Square London EC1V 0HB

Miss S Siviprasad Consultant Ophthalmologist, Kings College Hospital NHS Trust Denmark Hill London SE 5 9 RS 0203299 9000x 3542 senswathi@aol.com

Thank you for taking part in this study. This information sheet and a copy of any consent form you sign should be kept by you.

E1) EXCELL SHEETS comparing King's study patients to ETDRS and DRCRN patients

	DRCRN BASELINE (330EYES)	DRCRN 1YR (286)	DRCRN 2YR (272)	DRCRN 3YR	STUDY BASELINE	STUDY 1YR	STUDY 2YR	STUDY 3YR	STUDY 4 YR	STUDY 5YR	ETDRS 1YR	ETDRS 2YR	ETDRS 3YR
TOTAL COMPLETED	330	286	272	115		150	140	135	123	122	614(80%)	416(55%)	268(35%)
AGE	63			63	59								
WHITE	243(74%)				55(36%)								
BLACK	31(9%)				70(46%)								
ASIAN	7(2%)				25(16.6%)								
AMERICAN INDIAN	2(1%)												
HAWAIN	1(<1%)												
MULTI RACIAL	1(1%)												
UNKNOWN	6(2%)												
TYPE 1	14(4%)				15(10%)								
TYPE 2	316(96%)				135(90%)								
DURATION OF DM	15				13								
HBA1c median	7.5				9.52	9.16	9.22	8.81	8.88	8.11			
prior photocoagulation for DMO	198(60%)				NONE								
PHAKIC	262(79%)				138(92%)								
BASELINE VA	62				66								
MILD-MOD	186(58%)				123								
SEVERE	43(14%)				15								
PDR	88(28%)				9								
PRPC	53(16%)				3								

Mean(SD) change in VA	62	1+-16	2+-17	5+-17	66+-14	0+-14	-1+-15	-4+-16	-4+-16	-4+-17			
Median change in VA(IQR)25TH, 75TH percentile	62	3(-5,10)	5(-5,12)	8(-2 TO 15)	70	0	0	-5	-5	-5			
>=15 letter gain (15%)		14%	20%	30(26)		17(11.3%)	15(10.7%)	14(10.4%)	17(13.8%)	15(12.3%)			
10-14 letter gain		14%	14%	21(18)		13(8.6%)	11(7.86%)	10(7.4%)	3(2.43%)	5(4.1)			
5-9 letter gain		17%	17%	21(18)		19(12.6%)	20(14.3%)	16(11.9%)	13(10.56%)	8(6.56)			
no chage +- 4 letters		29%	22%	24(21)		58(38.6%)	38(27.2%)	26(19.3%)	24(19.51%)	30(24.6)			
5-9 letter loss		9%	9%	5(4)		13(8.6%)	16(11.4%)	17(12.6%)	18(14.63%)	17(13.94)			
10-14 letter loss		3%	6%	5(4)		11(7.3%)	16(11.4%)	13(9.6%)	12(9.75%)	16(13.12)			
>15 letter loss		14%	13%	9(8)		19(12.6%)	22(15.7%)	38(28.2%)	36(29.26%)	31(25.42)	5%	6%	0.1
ENDOPHTHALMITIS			0										
RETINAL DETACHEMENT			2										
RVO			3										
RAO			1										
AION			0										
VIT			31										
INCREASED IOP			25										
POAG			0										
GLAUCOMA SURGERY			0										
CATARACT SURGERY													
NO: LASERS ONCE			52(19%)							38(25%)			
2 TIMES			65(24%)							42(28%)			
3 TIMES			67(25%)							32(21%)			
4 TIMES			49(18							16(10%)			

	%)	
5 TIMES	28(10 %)	11(7%)
6 TIMES OR MORE	11(4%)	9(6%)
MEAN (SD)	2.9+- 1.4	2.66

E2) DATA SHEETS FOR KING'S LASER TREATED PATIENTS

Ethnicity	Eye	PRE LASER PROCEDURE	AGE AT FIRST LASER	Stage of DR: Mild/ moderate/ severe / PDR	time gap between macular and PRP	No: of macular lasers in 5 years	VA baseline	VA at 1st year	VA at 2nd year	VA at 3rd year	VA at 4th year	VA at 5th year	change in VA	censor	time	status	No: of eye appt in 5 years	No: of failed to attend appts in 5 yeas	VR surgery in 5 years	Cataract surgery in the 5 years:
3	L	2	32	2	6	2	85	75	60	60	75	75	-10	1	12	1 1	8	2	0	0
2	L	2	75	1		2	62	55	LOST				-7	0	12	1	9 LOST	4 LOST	0	0

1	L	2	78	1		1	75	75	60	60	45	DECEASED	-30	0	24	1		9+DECEASED	1+DECEASED	0	0
1	L	2	850	1	96	2	83	85	85	88	85	87	4	1	12	0		13	0	0	0
2	R	2	706	1		5	65	70	75	60	55	45	-20	1	121	1		20	8	0	0
1	L	2	606	1		1	58	38	88	70	70	82	-3	1	121	1	1	12	3	0	0
1	L	2	606	1		1	58	3	88	70	70	82	-3	1	121	1	1	12	3	0	0
1	L	2	65	1		3	45		35	35	40	40	-5	1	224	1	1	13	2	0	0
1	R	2	473	0		3	70	35	10	45	25	25	-45	1	121	1		20	1	1	1
1	L	2	471	0		1	57	58	70	70	73	73	-2	1	224	1	1	16	2	0	0
2	R	2	651			6	70	2	75	75	73	71	1	1	120			20	3	0	0
3	L	1	631	1		5	55	60	60	70	60	45	-10	1	601	1	1	16	2	0	1
1	R	2	949	2	9	3	57	75	75	60	60	75	0	1	360	0		23	4	1	0
1	R	2	949	2	30	3	57	75	75	60	60	75	0	1	360	0		23	4	1	0
2	L	2	553	0		3	53		35	55	53	33	-2	1	600	1	1	22	4	0	0

			6				7	5					-1		1						
1	R	1	5	3	-24	1	0	5	60	60	60	60	0	1	2	1	1	9		0	0
			5				6	6				DECEASE			1						
1	R	2	6	1		3	0	5	65	65		D	5	0	2	0		NA	N	0	0
			5				7	7							6						
3	L	2	0	1		2	5	5	70	70	70	75	0	1	0	0		16	4	0	0
			7				4	4							1						
3	R	2	3	1		3	5	5	40	40	40	40	-5	1	2	1	1			0	1
			4				6	6		LOS	LOS		1		2						
2	R	2	8	2	0	2	0	0	70	T	T	LOST	0	0	4	0		LOST	LOST		
													-2								
			6				6	7							2						
3	R	1	1	1		2	0	0	35	35	35	35	5	1	4	1		18	6	0	0
			7				6	3					1		6						
2	R	2	3	1		3	0	5	65	60	60	70	0	1	0	0	0	21	0	0	0
			6				4	7					1		1						
2	L	2	1	1		3	5	0	75	70	60	60	5	1	2	0	0	14	2	0	0
													-1								
			5				7	7							2						
2	L	2	7	2	42	6	5	5	60	55	55	65	0	1	4	1	1	26	5	0	0
			5				6	7							6						
2	L	1	8	1	62	2	0	0	70	65	75	55	-5	1	0	1		6	2	0	0
													-1								
			6				7	7							3						
1	R	2	5	3	0	2	0	5	70	60	60	60	0	1	6	1	1	30	3	0	0
			4				8	8							1						
2	R	2	6	3	0	1	0	5	82	84	LOST	LOST	4	1	2	0		11,LOST	2	0	0
			6				6	6							6						
2	R	2	2	1		6	0	0	60	45	65	60	0	1	0	0		20	5	1	0

1	L	2	57	1		3	70	85	85	85	85	85	15	1	12	0	0	15		2	0	0
1	L	2	58	1	36	2	70	75	70	60	70	0-1	1	12	0		19		3	0	0
2	R	2	54	1		6	75	70	75	70	60	15-1	1	60	1		16		1	0	0
2	L	2	41	1		2	70	75	75	70	35	35-1	1	60	1		20		8	0	0
2	R	2	77	1		5	80	70	69	75	70	85	5-1	1	60	0		30		10	0	0
2	L	2	62	1		2	85	82	70	77	LOST	LOST	5-1	0	24	1		12	LOST	LOST AFTER 3 YR	0	0
2	L	2	57	3	-48	1	75	80	70	85	85	55	0-2	1	60	1		14		4	0	0
2	L	1	80	1	35	3	65	45	50	50	50	50	5-1	1	12	1		7		1	0	0
2	R	2	75	1	19	5	60	20	35	20	35	35	5-2	1	12	1		35		3	1	1
1	R	2	67	1		4	80	75	85	75	75	62	8-1	1	12	1		20		3	0	0
2	L	2	59	2	96	3	45	45	45	45	45	55	0	1	60	0	0	19		4	0	0
2	L	2	61			2	34	40	35	35	35	35	0	1	60			10		2	0	0

2	R	2	16 5	1		5	57 60	0	75	75	75	75	5	1	06 0	0	18		2		0	0
2	R	2	70	1		3	70	0	55	60	LOST	LOST	-10	0	12	1	10,LOST		LOST			
1	R	2	71	1	24	4	70	0	60	55	55	50	-20	1	12	1	30		2		0	0
2	L	2	58	1	48	7	60	5	55	60	60	70	10	1	06 0	0	0 17		6		0	0
2	R	2	50	1	84	6	70	0	70	35	35	25	-45	1	06 0	1	15		3		0	0
1	R	2	71	1	24	4	70	0	60	55	55	50	-20	1	06 0	1	30		2		0	0
2	R	2	55	1	96	3	75	7 5	80	65	75	75	0	1	06 0	0	11		4 LOST FOR FOLLOW UP		0	0
2	L	2	64	1		5	75	70	60	60	70	55	-20	1	06 0	1	5		0		0	0
2	L	2	68	1	70	1	60	5 5	47	45	45	45	-15	1	06 0	1	11		2		0	0
2	R	2	60	3	-48	1	55	3 5	35	35	35	35	-20	1	06 0	1	12		5		0	0
3	R	2	59	1		3	90	8 7	55	84	83	73	-11	1	06 0	1	20		3		0	0

																		7								
			4				7	6											6							
3	L	2	7	1	85	2	0	2	84	77	65	70	0	1	0	0		10		2		0	0			
													-3													
			4				7	7											6							
2	R	2	4	2	70	1	0	0	70	60	55	35	5	1	0	1		10		4		0	0			
			5				4	6					3		6			12, LOST TO								
1	R	2	6	1	60	2	5	0	75	70	75	75	0	1	0	0	0	FU		4		0	0			
			5				8	7							6											
1	R	2	2	1	50	9	5	5	75	85	80	80	-5	1	0	1		31		7		0	0			
			5				8	7							6											
1	R	2	2	1	50	9	5	5	75	85	80	80	-5	1	0	1		31		7		0	0			
			4		11		7	8							6											
3	R	2	7	1	6	4	5	5	75	75	75	72	-3	1	0	1		17		3		0	0			
			5				5	5			LOS				3			7 UNTIL								
1	L	2	7	2		1	5	5	60	55	T	LOST	0	0	6	0		07/2004		N		1	0			
			5				5	5			LOS							7 UNTIL								
1	L	2	7	2		1	5	5	60	55	T	LOST	0	0				07/2004		N		1	0			
			6				7	7					1		6											
2	R	2	0	1		2	5	0	60	85	90	90	5	1	0	0	0	11		1		0	0			
			6				4	6					3		6											
2	L	1	8	1		3	5	0	60	75	45	75	0	1	0	0	0	16		2		0	0			
			6				7	7							6											
2	R	2	3	1		2	0	0	45	45	45	65	-5	1	0	1		5		1		0	0			
			5				7	7							6											
2	R	2	8	1		1	5	5	75	75	60	70	-5	1	0	1		12		2		0	0			
			5				6	7							6											
2	L	2	4	1		1	1	0	75	75	65	70	9	1	0	0		14		2		0	1			
			6				7	8							6											
3	L	2	1	1	48	2	0	5	85	75	75	75	5	1	0	0		21		2		0	0			

			6				7	7					-1		6												
2	L	2	7	1		5	5	5	75	75	70	60	5	1	0	1		28			5			0	0	
			6				6	4					-1		6												
2	L	2	7	1		6	0	5	35	25	35	45	5	1	0	1		29			4			0	1	
			5				8	8							6												
1	R	2	8	1		3	5	5	88	88	87	88	3	1	0	0		18			2			0	0	
			5				7	8					1		6												
3	L	2	6	1		2	0	5	75		85	85	5	1	0	0		18			4			0	0	
			5				7	8							6												
2	R	2	7	1		3	5	5	85	85	83	72	-3	1	0	1		23			4			0	0	
			4				7	7							6												
1	R	2	8	1		5	5	3	70	70	70	70	-5	1	0	1		20			2			0	0	
			4				7	7							6												
1	R	2	8	1		5	5	3	70	70	70	70	-5	1	0	1		20			2			0	0	
			7				6	6					1		6												
2	R	2	0	1		1	0	5	65	60	70	70	0	1	0	0	0	12			4			0	1	
			7				4	5					-2		6												
1	R	2	2	3	8	1	5	5	45	25	20	20	5	1	0	1		35			3			1	1	
			7				7	6					-1		2			17 IN 3YR,								
1	R	2	6	1		1	7	2	93	LOST			5	0	4	1		LOST FOR FU			5 AND LOST			0	0	
			6				7	7							1												
2	R	2	4	1		1	7	5	LOST				-2	0	2	1		4LOST			4 LOST			0	0	
			6				4	4			LOS		1		6												
2	R	1	1	1		2	5	5	55	55	T	LOST	0	0	0	0		LOST			LOST			0	0	
			6				7	7							6												
2	R	2	6	1		2	5	5	75	70	70	70	-5	1	0	1		10			0			0	0	

			7				7	6					-2		6					
1	R	2	1	1	24	4	0	0	60	55	55	50	0	1	0	1	30	2	0	0
			5				7	8							6					
1	L	2	4	1		1	5	0	75	75	75	75	0	1	0	0	7	2	0	0
			3				7	7							6					
2	R	2	8	1	20	3	5	4	55	50	75	75	0	1	0	0	17	0	0	0
			4				9	9							2			ONLY 2YR		
1	L	2	0	1		3	0	0	86	DNA	DNA	DNA	4	0	4	0	5, DNA	FU, DNA	0	0
			2				8	7					-2		6					
1	R	2	5	1	52	3	5	0	70	63	63	63	2	1	0	1	25	12	0	0
			4				7	8							6					
1	L	2	7	1		2	5	5	78	75	80	80	5	1	0	0	15	4	0	0
			5				4	5							6					
1	L	2	7	1		3	9	5	35	35	40	40	-9	1	0	1	10	0	0	0
			5				8	8					-1		6					
2	R	2	4	1		1	5	5	75	70	70	75	0	1	0	1	16	5	0	0
			5				4	5							1					
3	R	2	9	1		2	5	0	DNA			DNA	5	0	2	0	5 THEN DNA	2 THEN DNA		
			4				5	5					-2		6					
1	R	2	6	1	30	3	5	5	65	55	35	35	0	1	0	1	34	5	0	0
			4				5	5					-2							
1	R	2	6	1	30	3	5	5	65	55	35	35	0				34	5	0	0
			6				5								6					
1	R	2	4	1		2	5			60	55	47	-8	1	0	1	10	4	0	0
2	L	2	4	3	0	1	7	7	75	70	70	73	-2	1	6	1	16	2	1	0

1	R	2	75	1	27	3	54	55	70	75	75	65	2	1	06	0	0	25		1		0	0
2	R	2	65	1	12	1	50	55	46	55	70	40	-1	1	06	1		31		4		0	1
1	R	2	41	1	36	4	70	55	70	65	75	70	0	1	06	1		37		2		0	1
2	L	2	53	1		5	85	70	75	60	75	70	-1	1	06	1		18		3		0	0
3	L	2	60	1	36	4	29	40	60	47	55	55	2	1	06	0	0	25		4		0	0
3	R	2	68	1	36	3	76	50	65	68	70	70	-5	1	06	1		24		4		0	0
2	R	2	64	1		1	55	05	DNA				5	0	12	1		10		5		0	
2	L	2	62	1	10	2	37	50	75	50	65	65	3	1	06	0	0	18		1		0	0
3	L	2	63	1		2	56	60	60	55	60	50	-6	1	06	1		16		4		0	0
3	L	2	57	1		1	67	00	80	70	75	70	1	1	06	0	0	12		4		0	0
2	R	2	53	1		1	35	55	70	80	80	80	4	1	06	0	0	15		5		0	1
2	R	2	60	1		6	50	55	55	40	35	35	-1	1	06	1		14		2		0	0
1	L	2	53	3	0	2	87	00	35	20	20	20	-6	1	06	1		31		4		0	0

1	R	2	70	1		2	70	75	75	77	70	75	5	1	60	1	9	3		0	0
1	L	2	59	2	13	1	55	70	60	35	45	45	-10	1	60	1	6	2		0	0
1	L	2	49	1		3	55	65	DNA			DNA	-10	0	12	0	5 THEN DNA	3 DNA	THEN	0	0
2	R	2	64	1		2	70	70	75	60	60	60	-10	1	60	1	10	1		0	0
2	L	2	62	1	103	2	55	65	70	70	LOST	LOST	15	0	63	0	2, LOST	DNA 3YR	FOR	0	0
2	R	2	62	1	40	1	63	55	35	35	LOST	LOST	0	0	63	0	13, LOST	2		0	0
2	L	2	54	1	84	3	75	75	75	60	70	60	-15	1	60	1	28	2		0	0
1	L	2	83	1		2	35	55	25	20	LOST		-15	0	63	1	7 LOST TO FU	3		0	1
3	L	2	69	1		1	70	76	75	77	60	65	-5	1	60	1	14	3		0	0
1	R	2	53	1		2	70	75										LOST			
2	L	2	50	2	7	4	75	60	60	45	55	45	-30	1	60	1	31	0		0	1
1	L	2	52	1		1	85	70	70	90	90	85	0	1	60	0	12	5		0	0

2	L	2	83	1		1	65	75	70	70	70	70	5	1	60	0		11		4		0	0
3	L	1	72	1		1	70	75	70	70	70	70	0	1	60	0		18		6		0	0
1	R	2	58	1	36	4	80	85	80	80	60	60	-2	1	60	1		18		5		0	0
2	L	2	57	1	58	4	75	75	60	70	70	70	-5	1	60	1		12		3		0	0
2	R	2	64	1		2	60	85	85	85	85	90	3	1	60	0	0	14		N		0	0
1	L	1	65	1		2	77	80	75	75	75	70	-7	1	60	1		17		3		0	0
2	R	1	50	2		2	75	75	75	75	75	65	3	1	60	0		25		4		0	0
1	L	2	61	1		1	90	85	83	75	75	77	-13	1	60	1		15		2		0	0
1	L	2	44	2	8	2	70	80	75	LOST	LOST	LOST	5	0	36	0		19/ 3yr		2		0	0
2	R	2	55	1		2	55	LOST					0	0	12	0		7 LOST		LOST AFTER 2YR		0	0
2	L	2	69	1		2	62	35	25	LOST	LOST	LOST	-20	0	36	1		13 LOST		2, LOST AFTER 3YR		0	0
1	R	2	56	1		4	60	60	45	45	45	50	0	1	60	1		14		2		0	1
1	R	2	56	1		4	60	60	60	45	45	50	-11	1	60	1		14		2		0	1

			6				7	7					0		6							
2	R	2	1	1		3	5	5	70	75	75	75	0	1	0	0		9		N	0	0
			5				6	3							6							
3	R	2	4	1	14	1	0	5	60	70	65	60	0	1	0	0		29		4	1	0
			5				6	3							6							
3	R	2	5	1		1	0	5	60	70	65	60	0	1	0	0		29		4	1	0
			6				5	7					2		6							
2	L	1	5	1		1	5	0	70	75	75	75	0	1	0	0	0	12		2	0	0
			7				7															
2	R	2	4	3	6	1	0	0	0	0	0	0						22		2	1	1
			6				6	6					1		2			11 IN 3YR,		2, AND		
1	R	2	1	1		2	0	0	70	LOST			0	0	4	0		LOST FOR FU		LOST	0	0
			7				7	7							1							
2	L	2	1	1	17	4	5	5	LOST			LOST	0	0	2	0		3 AND LOST		2 AND LOST		
	Righ		7				8	8							6							
3	t	2	4	1		1	5	0	80	70	70	85	0	1	0	0		10		N	0	1
			7				8	8							6							
3	R	2	5	1		1	5	0	80	70	70	85	0	1	0	0		10		N	0	1
			7				8	8							6							
3	R	2	5	1		1	5	0	80	70	70	85	0	1	0	0		10		N	0	1
													-1									
			5		12		8	7	LOS		LOS				6							
3	R	2	0	1	6	3	5	5	T	75	T	75	0	0	0	1		LOST				
			7				3	2							6							
2	L	2	1	3	1	3	5	5	45	35	35	30	-5	1	0	1		20		3	0	0
			5				6	6							6							
2	L	2	1	1		1	0	0	60	70	65	65	5	1	0	0		9		2	0	0
			5				6	6					-		6							
1	R	2	6	1		4	0	0	60	45	45	50	1	1	0	1		19		2	0	1

			5				6	6					0- 1		6							
1	R	2	6	1		4	0	0	60	45	45	50	0	1	0	1	19		2		0	1
			6				7	7					4- 5		6							
1	L	1	6	1	54	3	5	0	65	65	30	30		1	0	1						
			6				7	7							6							
1	R	2	9	1	58	2	5	5	75	75	75	75	0	1	0	0						
			6																			
3	R	2	2	2	24	4																
			5				6	6							6							
2	L	2	9	1	27	4	0	0	55	60	64	60	0	1	0	0		13				
			6																			
2	R	2	8	1	34	4																
			7				4	5					1		6							
1	R	2	6	1	20	5	0	5	55	50	50	55	5	1	0	0	0	16				
			5				6	6					1		6							
3	R	2	2	2		2	0	0	55	5	76	76	6	1	0	0	0	16				
			4				6	6							1							
2	R	2	6	1	17	2	0	5	LOST	LOST	LOST	LOST	5	0	2	0		LOST				
			6				7	8			LOS				3							
2	R	2	2	1	36	3	0	5	65	65	T	LOST	5	0	6	0		LOST				
			6																			
2	L	2	6	1	40	3																
			4																			
3	R	2	6	1	50	3																
			5																			
3	R	2	5	1		3																
3	L	2	4	1	41	4																

8

E3) LIGHT ADAPTATION PATIENT DEMOGRAPHICS

					HbA1c		
TOTAL 42 PATIENTS CONSENTED		patient ID, CODE	AGE	TYPE DM	pre	3rd month	6th month
	AK	LE	59	2	7.3	7.1	6.4
	MM	LE	50	2	9.2	9.1	7.9
OTHER EYE AVASTIN TREATED	PD	RT	40	1	7	7.6	8.1
	BK	LE	71	2	6.1	6.1	6
	AM	LE	57	2	[redacted]		
	JC	LE	61	2	11.6	10.9	10.2
	PH	RT	61	2	8.1	8.2	7.7
	RB	RT	50	2	7.5	9.7	9.8
	BT	RT	55	2	6.4	7.7	7.4
	JN	LE	68	2	6.9	6.9	6.9
	OO	LE	42	2	[redacted]		
	WP	RT	41	2	10.8	10.8	10.8
	FI	RT	58	2	7.2	8	9.4
	JJ	LE	55	2	9.1	9.1	10.5
OTHER EYE PRPC	DS	RT	66	2	10.2	10.2	10.2
	BA	RT	52	2	10.3	10.4	10.4
	RS	RT	72	2	6.7	6.4	7.2
	HSK	RT	48	2	8.9	10.4	10.4
	AG	LE	49	2	7	7.7	7.7
	BP	LE	45	2	10.2	10.3	10.3
	CD	LE	75	2	8.2	7.3	8
	KM	LE	46	2	7.1	6.2	6.4
RE has undergone laser in 1st month	AM	LE	43	2	10	9.5	10.4
	SS	LE	45	2	8.6	8.3	8.3
	GU	LE	63	2	7.3	7.3	7.1
Left eye has undergone laser in 1st month/ ill health	BP	RT	51	2	9.3	9.3	9.5
	EB	RT	62	2	7.1	7.1	7.1

	MA	RT	44	2	10.1	10.1	10.1
	LD	RT	70	2	7.9	7.9	8.6
no 3rd m review	OP	RT	60	2	7.7	8.8	8.8
ill health	TC	LE	53	1	[redacted]		
	FN	LE	72	2			
non compliance first 3m	JR	LE	70		7.5	7.5	7.1
no 3rd m review	DM	RT	33	1	[redacted]		
stroke, hosp admission at 3m(laser control eye after 3rd m)	JS	RT	70	2	6.3	6.3	6.3

E4) CATEGORICAL CLASSIFICATION OF PROGRESSION

Progression in OCT SS analysis	progression in protan vision 6m	progression in tritan vision 6m	progression in contrast vision 6m	progression in mean MP1 6m	progression in vision 6m	progression in ZONE 1, MP1 6m	progression in ZONE 1 thickness 6m	progression in ZONE worst thickness 6m	Fundus photo progression month 6
1	0	0	1	0	0	0	0	0	1
1	0	0	0	0	0	0	0	0	0
1	1	1	0	0	0	0	1	1	1
1	1	1	0	0	0	0	0	0	0
1	0	1	0	0	0	0	1	1	1
0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	1	0	0	1
0	0	2	0	0	0	0	0	0	0
0	0	0	2	1	0	1	0	0	0
0	1	1	1	1	0	1	0	0	1
1	0	0	0	0	0	0	1	1	1
1	2	1	0	1	0	1	0	0	
1	0	2	1	0	0	0			1
1	0	0	0	0	0	0			1
2	0	0	1	0	0	0	0	0	0
1	0	1	0	0	0	0	0	1	0
1	0	1	0	0	0	0	0	1	1
1	0	0	0	0	0	0	1	0	1
1			0	0	0	0	0	0	2
0	0	1	0	0	0	1	1	0	0
2	0	0	0	0	0	0	2	2	2
1	0	2	0	0	1	0	0	1	1
1	1	0	2	0	0	0	0	0	2

0	1	0	0	0	0	0	2	0	2
2	0	1	0	0	1	0	2	0	0
0	0	0	0	0	0	0			0
0	0	0	1	0	0	0	0	0	2
1	0	0	0	0	1	0			0
1	0	0	0	0	0	0			2
0	2	0	0		2				
1	1	1	0	0	1	0	0	0	
1	0	0	0	0	0	0	0	1	2
1	0	1	0	1	1	0			
1	1	1	1	0	1	0	1	1	1
0	0	0	0	0	1	0	2	0	0
0	0	0	0		0		0	0	0
x									
0	0	1	0		0		0	0	0
1	0	0	0		0		0	0	1
0	1	1	0	0	1	0	0	0	2
0	0	0	0		2		0	0	2
0	0	0	0	0	0	0	0	0	2
0	0	0	2	0	2	0	0	0	1
0	1	1	0	0	0	1	0	0	0
0	0	0	2	0	0	1	0	0	0
1	0	0	0	0	0	1	0	0	1
0	2	1	0	0	0	0	0	0	
2	0	1	0	0	0	0	0	0	0
x									
0	0	0	0	0	1	0	0	0	1
0	2	0	0	0	0	0	2	0	0
0	0	0	2	0	0	0	0	0	2
0	0	0	2	0	0	2	0	0	2

2							0	0	2
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	2
x									
1	0	0	2	0	0	0	0	0	2
0	2	2	0	2	2	0	1	1	0
x									
2	0	0	2	0	0	0	2	0	0
0	0	0	0		2	0	0	0	0
0	2	0	0	0	0		0	0	0
0		1	0		2		0	0	0
0	0	0	0		2		0	0	
0	0	0	0	2	0	0	0	0	

F) KING'S RESEARCH & DEVELOPMENT APPROVAL LETTER

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12 March 2009

KH/WF

Full Research & Development Approval

R&D: KCH617
Title: Dark adaptation and diabetic retinopathy
REC Number: 08/H0808/196

Dear Miss Sivaprasad

Thank you for submitting your research project to the R&D Department. The project has now been approved by the Trust. Please quote the R&D registration number noted above in any communications with the R&D Department regarding your project.

Conditions of Approval:

- The Principal Investigator must notify R&D of the actual start and end date of the project.
- The Principal Investigator is responsible for ensuring that Data Protection Principles are observed throughout the course of the project.
- The agreed protocol must be followed. R&D must be notified of any changes to the protocol prior to implementation.
- The Principal Investigator and research team must have appropriate substantive or honorary contracts with the Trust. The Principal Investigator is responsible for ensuring that the team is covered, including new staff recruited to the study.
- If your study is a medicinal clinical trial all members of the research team must have completed GCP, Pharmacovigilance and Trial Master File training - please contact scott.vezina@kcl.ac.uk if training or annual updates are required.
- Please submit a copy of the progress report on the anniversary of the Ethics favourable opinion (sent via the CI)

Trust approval for the research is subject to the research being undertaken in line with the Department of Health's Research Governance Framework, and Trust policies relating to Research Governance.

INVESTOR IN PEOPLE

POSITIVE ABOUT DISABLED PEOPLE

The Research Governance Framework and details of you and your researchers responsibilities within this framework can be found on the Department of Health's website at:
http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_4108962

If appropriate it is recommended that you register with the Current Controlled Trials website; http://isrctn.org/

In line with the Research Governance Framework, your project may be randomly selected for monitoring for compliance against the standards set out in the Framework. For information, the Trust's process for the monitoring of projects and the associated guidance is available from the Trust's intranet or on request from the R&D Department. You will be notified by the R&D Department if and when your project has been selected as part of the monitoring process. No action is needed until that time.

Many thanks for registering your research project

Yours sincerely

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G) PUBLICATIONS, POSTERS, PRESENTATIONS

Presentations (oral, international)

- Light adaptation improves diabetic maculopathy. ARVO, May 2010

Posters (international)

- Intersession Repeatability of Visual Acuity, Colour Vision, Contrast Sensitivity, Retinal Sensitivity Thresholds and Retinal Thickness in Mild Diabetic Retinopathy. **Sreedhar B. Jyothi**, R Leung, Mathew Richardson, Elizabeth Pearce, Sobha Sivaprasad. ARVO 2011

- Effects of retinal neuronal thickness on contrast sensitivity in eyes with minimal diabetic retinopathy. Yi Fang Lee, **Sreedhar Jyothi**, Sobha Sivaprasad, Asia ARVO, Jan 2011

- Light adaptation delays diabetic maculopathy. Sobha Sivaprasad, **Sreedhar Jyothi**, Geoffrey Arden. EURETINA 2010

- Qualitative, Quantitative OCT Changes Following Laser Photocoagulation for Diabetic Macular Edema. R.V. Vemala, S. Koshy, **S. Jyothi**. ARVO 2010

- Visual Function Is Not Related To The Number And Location Of Microanuerysms In The Macula In Diabetic Retinopathy. Yi Fang Lee, **Sreedhar Jyothi**, Richard Leung, Elizabeth Pearce, Sobha Sivaprasad. ARVO 2010

- Long-term outcome following laser photocoagulation for diabetic macular oedema in Afro-Caribbean's. **S B Jyothi**, A Yandra, F Mubashar, T Adewoyin, S Sivaprasad. ARVO 2009

- Systemic factors in patients treated with macular laser for diabetic macular oedema in Type II diabetes. A Yandra, **S Jyothi**, F Mubashar, T Adewoyin, S Sivaprasad. ARVO 2009

- A Comparison of Functional and Structural Measures in identifying Progression of Diabetic Maculopathy. **S Jyothi**, S Sivaprasad. RCO 2011

- Microaneurysms count and distribution do not correlate with neuronal changes in early diabetic retinopathy, Yi Lee, **Sreedhar Jyothi**, Richard Leung, Elizabeth Pearce, Sobha Sivaprasad. RCO 2010

- The effects of laser photocoagulation on colour vision in patients with diabetic maculopathy. **S Jyothi**, Roger Wong, Geoffrey Arden, Sobha Sivaprasad. RCO 2010

Publications

- Five-year visual outcome following laser photocoagulation of diabetic macular oedema.
  S Jyothi, S Sivaprasad. Eye, EYE-10-960R, Aug 2011

- Prevention of dark adaptation causes regression of diabetic macular oedema.
  G Arden, **S Jyothi**, C Hogg, Y Li, S Sivaprasad. Eye, Oct 2011

Eye (2011) 25, 850–859

www.nature.com/eye

Medscape EDUCATION

Continuing Medical Education:

Five-year visual outcome following laser photocoagulation of diabetic macular oedema

S Jyothi and S Sivaprasad

Release date: 13 May 2011; Expiration date: 13 May 2012

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All other clinicians completing this activity will be issued a certificate of participation. To participate in this journal CME activity: (1) review the learning objectives and author disclosures; (2) study the education content; (3) take the post-test and/or complete the evaluation at **www.medscape.org/journal/eye**; (4) view/print certificate.

Learning objectives

Upon completion of this activity, participants will be able to:

1. Compare visual outcomes associated with laser photocoagulation treatment of diabetic macular oedema in a real-life, inner-city setting with those obtained in a clinical trial
2. Describe the effects of systemic risk factors on visual outcomes of laser photocoagulation treatment at 5 years in the real-life, inner-city setting
3. Describe the effects of other factors on visual outcomes of laser photocoagulation treatment at 5 years in the real-life, inner-city setting

Authors/Editors disclosure information

Andrew J Lotery has disclosed the following relevant financial relationships: Received grants for clinical research from: Novartis Pharmaceuticals Corporation. Served as an advisor or consultant for: Allergan, Inc.; Novartis Pharmaceuticals Corporation. Served as a speaker or member of a speakers bureau for: Novartis Pharmaceuticals Corporation.

Sreedhar Jyothi has disclosed no relevant financial relationships.

Sobha Sivaprasad has disclosed the following relevant financial relationships: Served as an advisor or consultant for: Novartis Pharmaceuticals Corporation; Pfizer Inc.; Allergan Inc. Served as a speaker or a member of a speakers bureau for: Novartis Pharmaceuticals Corporation; Pfizer Inc.; Allergan Inc. Received grants for clinical research from: Novartis Pharmaceuticals Corporation; Pfizer Inc.; Allergan Inc; Bayer HealthCare Pharmaceuticals

Journal CME author disclosure information

Laurie Barclay has disclosed no relevant financial relationships.

Five-year visual outcome following laser photocoagulation of diabetic macular oedema

S Jyothi and S Sivaprasad

CLINICAL STUDY

Abstract

Objective **To evaluate the 5-year visual outcome associated with laser photocoagulation treatment of diabetic macular oedema (DMO), and to investigate the relationship between systemic factors and visual outcomes in a real-life setting.**

Methods **The mean annual visual outcomes and systemic parameters of 100 consecutive subjects with type 2 diabetes who underwent the first session of focal/grid macular laser photocoagulation for clinically significant macular oedema between 2003 and 2004 were collected retrospectively and compared with the outcomes of the laser arm of the Diabetic Retinopathy Clinical Research Network (DRCRN trial comparing intravitreal triamcinolone acetonide injection with laser photocoagulation treatment for DMO). The primary outcome measures included the mean change in visual acuity (VA) in 5 years and the influence of systemic factors on final visual outcome.**

Results **The mean change in VA at 5 years was −5.23 in a real-life setting for an inner city population. The 3-year outcome was inferior to the clinical trial results with more people gaining vision (≥15 letter gain) in the DRCRN group compared with this cohort (26 *vs* 9%). Furthermore, three times more patients lost vision (>15 letter loss) in the real-life setting of this cohort compared with the clinical trial results of the DRCRN group (27 *vs* 8%, respectively).**

Conclusions **The visual outcomes and the control of systemic factors of patients with DMO in this cohort were inferior to those recruited for the clinical trial involving the DRCRN group.**

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Keywords: diabetic macular oedema; laser photocoagulation; ethnicity

Introduction

Diabetic maculopathy continues to be the leading cause of new onset vision loss among working age populations.[1] The Early Treatment of Diabetic Retinopathy Study (ETDRS) demonstrated that focal or grid laser photocoagulation reduced the risk of moderate visual loss in patients with clinically significant macular oedema (CSMO) by ~50% (from 24 to 12%) at 3 years, although visual acuity (VA) improvement was observed in <3% of cases, based on 15-letter gain at 3 years.[2] Despite the unsatisfactory outcomes, this treatment remains the gold standard of the treatment for CSMO. Indeed, recent clinical trials conducted by the Diabetic Retinopathy Clinical Research Network (DRCRN.net) indicate that the outcomes associated with macular laser treatment have improved significantly.[3,4] Advances in laser technology and optimisation of glycaemia and blood pressure (BP) control have been attributed to these beneficial outcomes.[5] Similarly, contemporary studies also suggest that the prevalence of diabetic retinopathy is decreasing when compared with the Wisconsin Epidemiologic Study of Diabetic Retinopathy published in 1984.[6] This decline in diabetic retinopathy prevalence is also thought to be because of the enhanced control of systemic factors.[7–9]

For more than a decade, the lessons from the UK Prospective Diabetes Study (UKPDS)[10] and the Diabetes Control and Complications Trial[11] studies have governed our clinical practise with regard to the management of diabetic

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This study was partly presented as a poster at ARVO 2009.

retinopathy and macular oedema (DMO). Strict glycaemic and BP control remain the most effective interventions to date. Given that contemporary clinical trials and prevalence data suggest an improvement in visual outcomes and better control of risk factors, we conducted a retrospective study to assess the 5-year visual outcome associated with macular laser photocoagulation (2003–2009) in a clinic-based setting catering to a multiethnic inner city population. We also determined the effect of systemic factors on visual outcomes to evaluate whether similar outcomes are obtained in real-life settings.

Methods

The protocol for this study was approved by the Chair of the institutional review board. The project was also registered in the Clinical Effectiveness Department of the institution. The study adhered to the tenets of the Declaration of Helsinki.

Study population

This study was carried out at the King's College Hospital, London, UK, where an established diabetic retinopathy screening programme caters to a multi-racial community with high levels of social and material deprivation. One-third of the total study population was drawn from Black and Ethnic minority groups. Individuals were graded as having diabetic maculopathy based on post-mydriatic two-field colour fundus photographs. These screen-positive patients were referred to the retinal clinics where a clinical examination and additional investigations (eg, fundus fluorescein angiography) were performed before laser photocoagulation. Optical Coherent tomography (OCT) was not available at baseline examinations.

Study design

Consecutive patients with type 2 diabetes and DMO who required their first macular laser photocoagulation in 2003–2004 were identified from the laser register. In bilateral cases, the first eye treated in each patient was included in the study. In cases in which both the eyes were treated during the same session, the eye with the poorer baseline VA was included. Patients who did not complete the 5-year follow-up were excluded from the study and the reasons for being lost to follow-up were recorded.

Laser photocoagulation

The focal/grid photocoagulation protocols used in the department mirror the DRCRN.net protocols (modified from the original ETDRS protocol).[1] In brief, the treatment was performed with a 514 nm green laser light Iridex Oculite GLx (Iridex Corp., Mountain View, CA, USA) with a spot size of 75–125 μm and an exposure time of 100 ms to obtain a light grey–white (just visible) burn and applied in a focal or grid pattern to cover the area of oedema.

The patients were reviewed every 4–6 months, unless they failed to attend an appointment. Laser treatment was repeated if clinical, angiographic and more recently, OCT evidence indicated a persistence of macular thickening. No distinction was made between focal or grid lasers in this study, because in clinical practise, many patients tend to have both on long-term follow-ups.

Visual acuity

VA was recorded using the Snellen VA charts in the early years, followed by the ETDRS charts at 2 m. As a result, all VA recordings were converted to ETDRS scores for this study. The VA examiners were not certified and the VA measurements were recorded in busy clinic settings. Under these circumstances, it is possible that the examiner did not spend enough time to encourage the patient to read as far as possible. As a result, the best-corrected VA may have been underestimated at times. The mean annual visual outcome was defined as the average of all VA measurements recorded per year.

Ocular and medical co-morbidity

All annual clinical data regarding ocular and medical history, including laboratory values, were obtained retrospectively from the electronic patient record, clinical files and laboratory records. Data collected that was related to systemic factors included age at first laser treatment, gender, ethnicity, length of duration of diabetes at baseline, date of initiating insulin therapy, average annual HbA1c levels, mean annual systolic and diastolic BP, number of anti-hypertensive medications at baseline and annually, average annual BMI, history of being on statins, history of cardiovascular co-morbidity, peripheral neuropathy, and foot ulcers.

Data collected that was related to ocular features included mean annual visual outcome, grade of diabetic retinopathy, date of cataract surgery (if carried out), number of macular laser treatments in 5 years, date of initiating pan-retinal photocoagulation (if required), history of any other surgical procedures including date, other ocular co-morbidity, number of retinal clinic

appointments in 5 years, and the number of appointments the subject failed to attend in 5 years.

Statistical analyses

The primary outcome measures in our patients (KCH cohort) included the mean annual change in visual outcomes up to 5 years; the 3 year outcomes were compared with the outcomes of the laser arm in the DRCRN randomised controlled study that compared intravitreal triamcinolone acetonide (IVTA) with laser photocoagulation for DMO.[12] The last observation carried forward method was used to assign 45 missing values over the 5-year study period. Data were expressed as percentages, mean values (with standard deviations) or median values. In the univariate analyses, we compared each of the variables using *t*-tests, Mann–Whitney *U*-test and Fisher's exact test where appropriate. After the univariate analysis, a multivariate logistic regression model of patient characteristics and outcomes was performed to identify the clinical variables associated with gain of vision (ie, losing $\leq$5 ETDRS letters). To correct for multiple comparisons, results were only included in the multivariate analyses when the corresponding $P<0.01$.[1]

Results

The baseline characteristics of the study cohort are summarised and compared with the DRCRN study population in Table 1. The mean age of the patients at study baseline was 68.8 years (range 38–91 years), with 47 (31%) female and 53 (69%) male patients. A total of 201 clinical notes were screened to identify patients who met the criteria for enrolment; causes for exclusion included lack of adequate follow-up ($n=54$), lost to follow-up ($n=32$), and mortality ($n=15$).

Visual outcomes

The mean change in VA at 3 years was −4.15 ETDRS letters in the KCH cohort relative to a gain of 5 ETDRS letters in the DRCRN study. In the first year, the percentages of gainers (ie, patients who experienced a loss of $\leq$5 ETDRS letters) were similar in both groups (73% in the KCH cohort *vs* 74% in the DRCRN laser group). However, by the third year, only 50% of the KCH group patients were gainers compared with 83% in the DRCRN laser group. The proportion of gainers in the KCH cohort was relatively similar from the third to fifth years after the first laser treatment (47–50%; Table 2). Only 1 out of 10 KCH cohort members gained $\geq$15 ETDRS letters at year 1, and this result was maintained to year 5. However, in the DRCRN laser group, the number of patients that gained $\geq$15 ETDRS letters nearly doubled from 14% in the first year to 26% in the third year. The results with the KCH cohort are superior to those of the ETDRS study,[2] in which only 3% gained $\geq$15 ETDRS letters. When we considered the proportion of patients with moderate visual loss at 3 years (loss of $\geq$15 ETDRS letters), the outcomes with the KCH cohort are inferior (27%) to those of the DRCRN laser group (8%). Taken together, the results of these comparisons show that the visual outcomes of the KCH cohort are inferior to the visual outcomes of the laser group in the contemporary DRCRN study.

Table 1 Baseline characteristics of the KCH cohort relative to the DRCRN laser group[12]

	DRCRN laser arm	*KCH cohort*	*P-value*
Number of patients	115	100	
Mean age at first laser in years	63	59	
Duration of diabetes at baseline in years	15	13.53	
Mean HbA1c	7.5%	8.5%	
Before laser at baseline	60%	None	<0.0001
Baseline VA	62	67	
Phakic at baseline	79%	91%	
Ethnicity at baseline			
White	74%	38%	<0.0001
Black	9%	47%	<0.0001
Asian	2%	13%	0.0055
Others	15%	2%	0.0015
Type of diabetes			
Type 1	4%	0%	0.1
Type 2	96%	100%	
Retinopathy status at baseline			
Mild	58%	85%	<0.0001
Mod	14%	6%	
Severe	28%	6%	
PDR	16%	3%	

Abbreviations: DRCRN, Diabetic Retinopathy Clinical Research Network; HbA1C, glycosylated haemoglobin; PDR, proliferative diabetic retinopathy; VA, visual acuity.

Mean number of laser treatments

The mean number of laser treatments over the 5-year study period for the KCH cohort was 2.74 ± 1.6. Table 3 shows the number of laser treatments for the KCH cohort compared with the DRCRN laser group. The mean number of laser treatments performed was less for the KCH cohort, and more patients in the KCH cohort had only one laser session compared with the DRCRN laser group, despite the fact that 60% of the DRCRN group had previous laser treatment and 13% of the DRCRN

Table 2 Annual mean visual outcomes of the KCH cohort compared with the DRCRN laser group outcomes[12]

Changes in VA	*KCH first year*	*DRCRN first year*	*KCH second year*	*DRCRN second year*	*KCH third year*	*DRCRN third year*	*KCH fourth year*	*KCH fifth year*
Mean	−0.48 ± 11.74	1 ± 16	−2.08 ± 14.62	2 ± 17	−4.15 ± 15.2	5 ± 17	−4.03 ± 15.34	−5.23 ± 17.2
Median (95% CI)	0 (−2.7, 1.8)	3 (−5, 10)	0 (−4.9, 0.79)	5 (−5, 12)	−4 (−7, −1)	8 (−2, 15)	−5 (−7, −1)	−5 (−8.6, −1.8)
≥15 letter gain (%)	10	14	10	20	9	26	13	12
10–14 letter gain (%)	11	14	6	14	5	18	2	4
5–9 letter gain (%)	10	17	18	17	14	18	12	9
No change ± 4 letters (%)	42	29	27	22	22	21	20	22
5–9 letter loss (%)	6	9	8	9	12	4	17	16
10–14 letter loss (%)	7	3	10	6	11	4	8	10
>15 letter loss (%)	14	14	21	13	27	8	28	27

Abbreviations: DRCRN, Diabetic Retinopathy Clinical Research Network; VA, visual acuity.

Table 3 Number of laser treatments in the KCH cohort compared with the DRCRN laser group

Number of laser treatments	*DRCRN laser group (third year)*	*KCH cohort (third year)*	*KCH cohort (fifth year)*
Once	19	32	23
Two sessions	24	28	32
Three sessions	25	20	21
Four sessions	18	5	8
Five sessions	10	7	8
Six sessions or more	4	8	8
Mean laser sessions	2.9 ± 1.4	2.54 ± 2.0	2.74 ± 1.6

Abbreviation: DRCRN, Diabetic Retinopathy Clinical Research Network.

Table 4 Changes in HbA1C and blood pressure in KCH cohort over the 5-year study period

	KCH baseline	*KCH year 1*	*KCH year 2*	*KCH year 3*	*KCH year 4*	*KCH year 5*
HbA1C Mean ± SD (range)	9.25 ± 1.99 (5.7–15.4)	9.17 ± 2.09 (5.6–18.6)	9.4 ± 2.06 (5.8–15.6)	8.82 ± 1.87 (4.4–13.5)	8.85 ± 1.82 (5.5–16.8)	8.7 ± 1.81 (6.2–16.8)
Systolic BP Mean ± SD (range)	143 ± 23.37 (93–234)	142 ± 21.31 (94–195)	144 ± 22.36 (82–200)	142 ± 19.70 (95–190)	140 ± 21.76 (92–200)	141 ± 21.57 (84–204)
Diastolic BP Mean ± SD (range)	80 ± 11.56 (50–122)	79 ± 11.88 (52–110)	78 ± 12.15 (43–105)	77 ± 10.7 (46–105)	75 ± 11.51 (50–108)	77 ± 11.59 (50–108)

Abbreviations: BP, blood pressure; HbA1c, glycosylated haemoglobin.

group had additional treatments other than laser (eg, IVTA and bevacizumab). All of the patients in the KCH cohort were treatment naive and none of the KCH cohort patients received any additional intravitreal treatments. Notably, the proportion of patients having four or more laser sessions in the KCH group was less than that in the DRCRN group.

Influence of systemic factors on visual outcomes at 5 years

Table 4 shows the mean annual changes in HbA1C and systolic and diastolic BP in the KCH cohort over 5 years in the current era of improved glycaemia and BP control relative to the DRCRN cohort. Although the mean HbA1C and BP values in the KCH cohort improved slowly over the 5-year study period, the overall control of risk factors for the KCH cohort was inferior to the baseline data for the DRCRN laser group.

Univariate analyses of the known risk factors are shown in Table 5. Gainers were defined as those who lost ≤5 ETDRS letters; the rest were termed losers. Insulin users, BMI ≥25, better baseline VA (≥55 ETDRS letters), number of laser treatments (a surrogate marker of severity of DMO), and more number of failed appointments were associated with poorer visual

Table 5 Univariate analysis of the prognostic systemic and ocular factors for gain in vision after macular laser treatment for DMO

	Gainers (n = *47*)	*Losers* (n = *53*)	P-*value*
Systemic factors			
Age at baseline (years)			
<65	26	11	0.8
≥65	35	28	0.3
Ethnic groups			
Caucasians	20	17	0.3
Non-Caucasians	27	36	
Gender			
Male	25	28	0.2
Female	22	25	
Duration of diabetes (years)			
<15	26	37	0.9
≥15	21	16	0.4
Diabetic medications			
Oral	11	10	0.009
Insulin/oral + insulin	36	43	
Time to start of insulin			
Before first laser	24	22	0.9
During the 5 years	7	12	0.8
Baseline HbA1C			
<7.5	8	13	0.5
≥7.5	39	40	0.9
Baseline systolic BP, mm Hg			
<140	21	24	0.4
≥140	26	29	0.7
Baseline diastolic BP, mm Hg			
<90	36	41	0.9
≥90	11	12	0.3
Number of antihypertensives at end of follow-up			
0–2	22	33	0.09
≥3	25	20	
Baseline BMI			
<25	9	6	0.7
≥25	38	47	0.0006
Ocular factors			
Baseline VA (ETDRS letters)			
<55	10	6	0.001
≥55	20	23	0.4
Lens status: phakic	44	48	
Previous pseudophakia	3	5	0.2
Pseudophakia during study	2	5	
DR status at baseline			
Non-PDR	43	48	0.3
PDR	4	5	
Number of macular laser treatments			
1–3	39	37	0.008
>3	8	16	
Number of clinic appointments			
Mean	19.23	19.6	
Range	4–34	3–39	
Number of failed clinic appointments			
Mean	3.02	3.75	0.009
Range	0–7	0–12	

Abbreviations: BMI, body mass index; BP, blood pressure; DM, diabetes mellitus; DMO, diabetic macular oedema; ETDRS, early treatment diabetic retinopathy study; HT, hypertension; PRP, pan-retinal photocoagulation.
Gainers: loss of <5 ETDRS letters; losers: loss of ≥5 ETDRS letters.

Table 6 Multivariate model for visual outcome at 5 years

Factors with P<0.01 in univariate	*95% CI*	P-*value*
Type of anti-diabetics	−8.732 to −0.9495	0.0152
Baseline BMI	−0.2670 to 1.059	0.2378
Baseline VA	−0.6238 to −0.1634	0.0010
No. of macular lasers	−3.109 to 0.7052	0.2132
No. of failed appointment	−3.191 to 0.1071	0.0661

Abbreviations: BMI, body mass index; CI, confidence interval; VA, visual acuity.

outcome. However, the multivariate model showed that better baseline VA and insulin users were the only poor prognostic indicators (Table 6).

Discussion

Laser photocoagulation remains the standard treatment for patients with CSMO. The main objective of laser treatment is to prevent visual loss, rather than improve vision. Nevertheless, contemporary studies on laser photocoagulation for CSMO indicate that the visual outcomes with macular laser treatment are much better than those obtained with the ETDRS study with ∼1 in 4 gaining ≥15 ETDRS letters by 3 years.[1,13,14] The suggested reasons for this improvement include better glycaemic and BP control and perhaps early detection and prompt treatment of cases compared with a decade ago. However, our study in a clinical setting catering to a multiracial inner city population shows that the long-term results (3–5 years) are inferior to those obtained in clinical trials with ∼12% showing improved vision and 26% suffering moderate vision loss at 5 years.

We assessed a number of factors that may determine the poorer outcome. These factors included demographics, ocular and systemic factors and issues associated with healthcare provisions. Compared with the DRCRN study (baseline data comparing IVTA to laser for DMO), the KCH cohort was younger and contained more ethnic minority groups. But the treatment outcomes for Caucasians and other ethnic groups were not dissimilar ruling out inequalities to access to health care (data not shown).

The mean HbA1C of our group was 8.5% compared with 7.5% in the DRCRN group. HbA1c levels of ≥ 8 are associated with an increased risk of macular oedema, irrespective of the ethnic group.[15] In a recent report in our population, we found that the risk of diabetic maculopathy independent of the ethnic group is significantly higher in subjects registered with family practices with the lowest quartile of HbA1c achievement.[16,17]

However, the present study results reflect those of the DRCRN group, indicating that the levels of HbA1C do not influence the outcomes of macular laser treatment. Thus, decreasing HbA1C levels is more important with regard to the prevention of maculopathy than with maculopathy treatment. This finding suggests that over time, other factors such as increased vascular endothelial growth factor levels may dominate the course of the disease.[18] Patients in the KCH cohort also had higher systolic and diastolic BP compared with the DRCRN group. Again, this difference may be explained in part by the differential susceptibility of the African–Caribbean group to high BP. However, unlike the ETDRS study, the DRCRN study reported that baseline systolic BP and mean arterial BP did not influence VA outcomes. Despite the higher BP in the KCH cohort, univariate analyses did not reveal BP as a predictive factor. As discussed above with regard to HbA1C, epidemiological studies and clinical trials strongly support hypertension as an important modifiable risk factor for diabetic retinopathy.[19] In the UKPDS study, tight BP control reduced the risk of retinopathy progression by about one-third, visual loss by one-half and the need for laser treatment by one-third in patients with type 2 diabetes. Similarly, the EUCLID study,[20] DIRECT study,[21] and RASS study[22] show positive outcomes for anti-hypertensives on retinopathy risk. However, these are risk reduction strategies for the development and progression of DR. Although both HbA1C and BP must be optimally controlled to decrease the rate of incident DR and maculopathy, they do not appear to influence laser treatment outcomes as shown in the current study and based on the analysis of the DRCRN group.[1]

Owing to the large number of variables evaluated, we only considered associations with a $P<0.01$ to be significant. Poor prognostic indicators included insulin users, less number of laser treatment sessions, more number of missed clinic appointments, better baseline VA, and BMI ≥ 25. Nevertheless, only a few of the variables met a $P<0.05$ value threshold in multivariate analyses; they were the univariants, being on insulin medication and baseline VA. Similar to the analyses of the DRCRN group,[1] we found that visual improvement was better in eyes with poorer baseline VA (<55 ETDRS letters). These types of ceiling and floor effects have been reported for treatment outcomes associated with both diabetic maculopathy and age-related macular degeneration.[1,23] The duration of oedema may be an important determinant of final visual outcomes, but this factor was not analysed directly in the current study. Nevertheless, the poorer results in year 3–5 may serve as a surrogate marker of chronicity of disease.

Despite the fact that all our patients were treatment naive at baseline, the mean number of laser applications was only 2.7 at 5 years compared with 2.9 at 3 years in the DRCRN group. Although it did not reach a significant level in the multivariate model, the number of laser applications is an important factor that may have influenced our outcomes. The high threshold among retinal specialists to perform more lasers when 2–3 attempts have not shown a positive response should change based on recent data reported by the DRCRN indicating that the probability of improvement of eyes treated previously with laser ≥ 3 times had a similar chance of VA improvement as eyes that had not had previous laser treatment.[4] Taken together, these findings suggest that it is useful to proceed with further laser treatment if there is sufficient space to apply more burns. It is also important to note that the response to laser treatment is slow, and that persistent oedema after one to two laser treatments should not deter physicians from re-treating.

Another significant problem in the real-life setting of urban populations is the lack of awareness of diabetic retinopathy and its associated complications. In all, 22% of the patients in our recent study of urban populations failed to attend screening appointments[16] with the highest non-attendance reported among 18–34 year olds. In this study, $\sim 50\%$ of the subjects were not followed-up regularly, and 16% were lost to follow-up. Therefore, our results may be worse than reported if the outcomes for the lost patients were known. Our current screening and treatment guidelines ensure that patients with sight-threatening disease are promptly referred and treated. However, the major challenge of providing timely monitoring and treatment appointments for these patients remains unaddressed.

The strength of this study is that it included the largest number of patients with DMO who had macular laser

treatments in real-life settings with long-term follow-up, thereby allowing the results to be compared with outcomes from contemporary clinical trial results. However, a limitation of this study is its retrospective nature. Despite the fact that we recruited consecutive patients with 5-year follow-up, ~50% of the patients did not complete the 5-year follow-up or did not have at least one annual follow-up visit during this time period. Therefore, we can only postulate that the results may be inferior to our present data if all patients would have been followed. Finally, we did not differentiate between focal and diffuse macular oedema in this study, as angiograms were not available in all cases.

In summary, this study shows that retinal specialists should contemplate further laser treatments in patients with persistent oedema despite potential initial non-responsiveness to laser treatment. Rigorous measures should be initiated to ensure timely follow-up to avoid non-attendance and resultant loss of vision of these high risk individuals.

Summary

What was known before

- Long-term laser photocoagulation outcome for diabetic macular oedema in clinical trials setting.

What this study adds

- Long-term laser photocoagulation outcome for diabetic macular oedema in real-life settings.

Conflict of interest

Sobha Sivaprasad has received travel grants, research grants, and attended advisory board meetings for Pfizer, Novartis, and Allergan.

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Fast Track Paper

CLINICAL STUDY

Regression of early diabetic macular oedema is associated with prevention of dark adaptation

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Abstract

Hypothesis **Dark-adapted rods consume oxygen at high rates and light adaptation decreases this oxygen burden and can have therapeutic effects on diabetic macular oedema (DMO).**

Methods **Patients with mild non-proliferative diabetic retinopathy (DR) and early, untreated non-sight-threatening DMO slept for 6 months wearing masks that illuminated the eyelid of one closed eye by 505 nm light. Exclusion criteria were any concomitant eye disease, DR >ETDRS grade 35, and other systemic diseases. Primary outcome: change of OCT retinal thickness in the local region where oedema was present.**

Results **A total of 34 out of 40 patients completed the study. Mean baseline OCT macular cube thickness was equivalent for study and fellow eyes. But study eyes had a greater mean thickness in the central subfield zone 1 ($282 \pm 53\ \mu m$) *vs* ($256 \pm 19\ \mu m$) the fellow eyes. Twenty-eight study eyes showed intraretinal cysts compared with nine in the fellow eyes. At 6 months, only 19 study eyes had cysts while cysts were seen in 20 fellow eyes. After 6 months, the worst affected ETDRS zone and the central subfield zone 1 reduced in thickness in study eyes only by $12\ \mu m$ (95% CI 20 to −7, $P = 0.01$). The secondary outcomes of change in visual acuity, achromatic contrast sensitivity, and microperimetric thresholds improved significantly in study eyes and deteriorated in fellow eyes.**

Conclusions **Sleeping in dim light that can keep rods light adapted may reverse the changes of DMO.**

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Introduction

Recent clinical trials have shown that intravitreal injections of inhibitors of vascular endothelial growth factor (VEGF) can improve visual function and retinal morphology in patients with diabetic retinopathy (DR), and diabetic macular oedema (DMO).[1–4] In such patients, there may be widespread loss of retinal capillaries that lead to retinal hypoxia, which is known as the most potent stimulus for VEGF upregulation. VEGF has properties that could cause the vascular damage observed in DR and DMO.[5] But in cat and man there is evidence of hypoxia before any capillary dropout. In the cat,[6,7] the evidence is from direct measurement of intraretinal pO_2 using oxygen microelectrodes. In man, before any vasculopathy can be demonstrated, dark adaptation is incomplete,[8] colour discrimination sensitivity[9] and achromatic contrast sensitivity decline,[10] and decrease of the oscillatory potentials can be seen.[11] These early changes are likely to be associated with hypoxia because they are partially, rapidly, and transiently reversed by oxygen inhalation. The cause of these changes remains unclear. It is to this problem that the present work is directed. One fundamental observation is that the vascular changes seen in retinal vessels do not occur in brain tissue although the tissues have similar origins. There must therefore be a specific retinal peculiarity causing DR.[12] The main difference between the brain and retina—that is the presence of photoreceptors—may be responsible for the retinal susceptibility. There is

considerable evidence to support this view.[13–18] These and other observations have led to a different proposal about the pathophysiology of DR.[19,20–22] The oxygen demand of rods, in the outer retina, is much increased in darkness[6,7,13,23–25] and this in turn causes a reduction in inner retinal oxygen tension.[11,25] It has been suggested that the increasing inner retinal hypoxia in diabetes causes the upregulation of cytokines, most importantly VEGF, to a degree that produces damage to small retinal vessels, thus reducing local capillary blood flow and increasing hypoxia in a vicious circle. This hypothesis predicts that prevention of dark adaptation should reduce the rate of progression of DMO[22–24] because outer retinal hypoxia increases considerably in dark adaptation.[6]

A phase I clinical trial involving 12 patients with mild non-proliferative DR showed that trans-eyelid illumination of the retina during sleep was acceptable by patients, had no reported ill effects, and also improved DR and visual function.[26] In the light of this finding, we have conducted a further investigation on patients with early DMO. We hypothesised that DMO would be as susceptible to this intervention as had DR.

Materials and methods

This study was conducted in accordance with the Declaration of Helsinki, and approval was obtained from King's College Hospital Ethics Committee (ISRCTN34037927; R&D 08/H0808/198). Written informed consent was obtained from all participants.

Subjects were recruited from the Medical Retina clinics of King's College Hospital NHS Foundation Trust. The inclusion criteria were: (1) clinical evidence of focal macular thickening that was clinically nonsignificant within two disc diameters of the centre of the fovea or too close to the foveal avascular zone for laser treatment; (2) an Early Treatment Diabetic Retinopathy Study (ETDRS) retinopathy severity level between 20 and 35; (3) a BCVA of 60 or more letters (ETDRS 4 m protocol); (4) no history of scatter (panretinal) or focal/grid photocoagulation for DR; and (5) no evidence of other ocular pathology that could interfere with ocular examination and visual acuity assessment during the 6-month study period. The exclusion criteria were: (1) clinically significant macular oedema with visual acuity of <70 letters that was amenable to laser photocoagulation at baseline according to ETDRS guidelines; (2) visual acuity reduction that could not be attributable to DR; and (3) any other concomitant ocular or systemic conditions that could influence the natural course of DR.

Patients who, during the study, required additional treatment in the non-study eye due to deterioration in DMO are not included in the statistical analyses.

Lightmasks—construction and design

A plastic cup of transparent silicone rubber contained four light-emitting diodes (Nichia NESE021T rank E, Nichia Corporation, Tokushima, Japan). Its concave surface fitted on the closed lid of either a left or right eye. In preliminary experiments (not reported) similar to those described in Arden *et al*[14], we determined that the 505 nm light used increased rod threshold by $\sim$3 log units before the increment threshold for cones increased. The calculated light intensity at the retina was 2 scotopic Trolands, which would cause a considerable reduction in the rod dark current.[24] The device was driven by a rechargeable battery (Panasonic UL23300, Panasonic Corporation, Osaka, Japan) connected to a charge-pump chip (Max 1573, Maxim Integrated Products, Inc. Sunnyvale, CA, USA). The battery was recharged each morning. A train of short-light pulses, with variable mark–space ratio, regulated the light output. Each LED drew $\sim 200\,\mu$A current. The printed circuit board was enclosed in a thick cotton cover, held against the eyes by an elastic headband.

Study design

Eligible patients were provided with the information leaflet, and used the eye mask for a trial period of 1 week before consenting to the study. Following the baseline visit, patients returned at 3 and 6 months for all tests except microperimetry (see below). Patients were contacted in between the visits to reinforce compliance and returned to the study centre if they had any trouble with the masks. Patients were directly questioned about any sleep disturbance but formal sleep questionnaires were not used. The technicians conducting OCT, and psychophysical tests were masked of the study eyes while the physicians and patients were not. Randomisation was not attempted because many patients presented with the diagnosis of unilateral disease.

Best-corrected distance acuity was measured using ETDRS charts (Lighthouse Low Vision Products, New York, NY, USA) at 4 m. The Pelli–Robson contrast sensitivity test was performed using two charts (one for each eye) at a distance of 1 m. Microperimetry was performed with the automated microperimeter (MP1; Nidek Technologies, Padova, Italy) in selected patients who agreed to this examination. The stimulus used was the Goldmann III spot size of 200-ms duration, attenuation 0–20 dB. The foveal and mean retinal sensitivity was recorded. Standard 2-view fundus photographs were taken. Spectral-domain OCT was performed using the Macular Cube 512×128 scanning protocols (Cirrus, Carl Zeiss Meditec AG, Jena, Germany). Intraretinal cysts were classified as present or

absent at baseline. As retinal swelling was localised, retinal thickness was also recorded for the nine separate ETDRS zones. Additional treatment (macular laser photocoagulation) was given, if required.

Safety assessments

All patients were asked if they had any complaints, or discomfort, or any sleep disturbance or mood alterations or changes in wakefulness during the day. These were recorded as adverse events.

Statistical analyses

Significance for continuous variables was estimated using two-tailed paired *t*-tests. The null hypothesis was rejected for $P < 0.05$. The Fisher exact test was also employed.

Study design
By treating only one eye, we ensured that important variables were equivalent in treated and non-treated eyes (eg, HbA1c, blood pressure, age, sex, smoking, etc). The number of study eyes with intraretinal cysts exceeded those in the fellow eyes, and there was a greater thickness of zone 1 in OCT measurements in study eyes than fellow eyes. For this reason, a longitudinal analysis of changes in eyes has been carried out for study and fellow eyes independently, in addition to the study–fellow eye comparison.

Outcomes
This to the best of our knowledge is the first publication in which light masks have been used to treat DMO. It is also the first publication to describe changes in OCT findings with time in patients undergoing treatment for early disease. For this reason, we defined the outcome as a reduction in local retinal swelling, recording the thickness of each of the nine ETDRS regions in the OCT analyses. We here report on zone 1 thickness and the thickness in the most swollen zone, which was always in an intermediate zone (zones 2,3,4, and 5). We also report on the thickness of the zone opposite the zone of maximum thickness, the 'mirror zone'. If the maximum thickness occurred in zone 2, the mirror zone was 4, if 3, 5 and so on. The mirror zone was thus on the same eccentricity as the zone of maximum swelling but as far away from the swelling as possible. The rationale is that it provides an additional control. Reduction of thickness in one zone might occur as a temporary regression, accompanied by concurrent worsening in another: the fact this did (or did not) occur in a zone where swelling was minimal is therefore of interest, as is the change—or absence of change—in untreated eyes examined using the same method of analysis. The psychophysical tests we used were all standard, and results were obtained in exactly the same way as used by previous investigators. We initially measured microperimetric thresholds in the ETDRS zones, but this test was abandoned after 3 months because participants found the test too difficult.

Results

The study enrolled 5 patients with type I (12%) and 35 with type II diabetes mellitus. The mean age was 56 years (range 33–75 years). The mean baseline glycated haemoglobin (HbA_{1c}) was 8.21% (range 6.0–11.6%) and altered at 6 months by -0.17 ± 0.88 (SD). All investigations were carried out between 1400 and 1700 hours.

Dropouts

Out of the 40 recruited, 6 failed to complete the study; 1 dropped out after 3 months due to inability to keep hospital appointments, and 1 failed to use the lightmask in the first 3 months. Four discontinued because of unrelated ill health. A further four patients were excluded from paired analyses because the unilluminated fellow eyes received laser or intravitreal bevacizumab for progression of DMO during the study. None of the illuminated eyes required such treatment during the study. Thus, a total of 34 patients were included in the analyses of the changes in the illuminated eyes at 6 months. In two cases, 3-month OCT measurements were not done.

Adverse effects

None of the recruited subjects reported any difficulty in wearing the masks, or any difficulty in sleeping, or mood alteration.

Fundus photographs did not change over 6 months, and are not reported upon further.

OCT results

At baseline, the mean macular cube thickness was nearly equal for study and fellow eyes (282 ± 51 μm *vs* 278 ± 25 μm), and changed insignificantly during the study (279 ± 16 μm *vs* 279 ± 22 μm) because the DMO was localised as can be seen in Figure 2.

However, the mean central subfield (zone 1) thickness was higher in the study eyes compared with the fellow eyes. Also, the number of patients in whom an intraretinal cyst was seen in OCT transverse section was greater for study eyes than for fellow eyes (28 in study eyes and 9 in fellow eyes). Representative samples of the morphological regression of intraretinal cysts in the study eyes over the 6 months are given in Figure 1 for

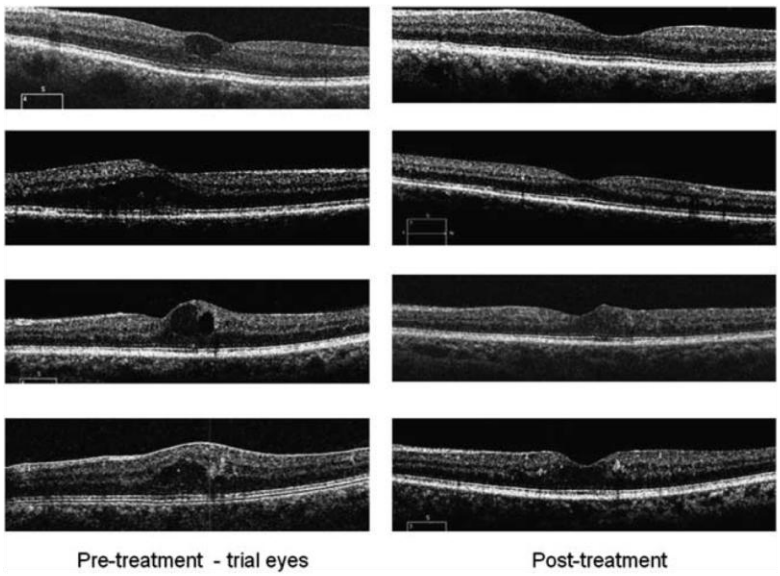

Figure 1 Cross-sectional OCT views horizontally through the macula from some representative patients study eyes. In each case, the characteristic cysts have decreased over the course of the study.

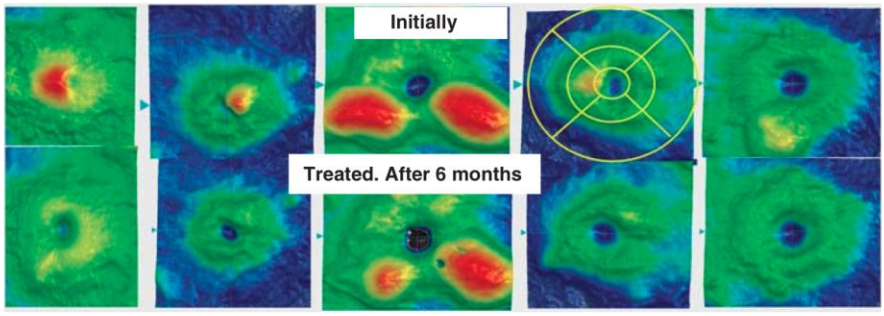

Figure 2 Computed 3-D OCT false colour images in representative patients, showing changes in retinal thickness. Blue and dark green colours are within normal limits, and green, yellow, red, pink, and white represent regions of increased thickness, which varies between patients. Swelling diminishes during the trial. Quantitation of such results was achieved by using the separation of different zones, included in the OCT report, which are indicated for one eye, in the figure. These are based on the ETDRS zones, and results are given in the tables. Zone 1 is the subfoveal zone for the left-handed patient, the zone measured was zone 2, and measurements are also presented from zone 4, opposite it, which is called the 'mirror zone'. For the right-most pair of images, measurements would be taken from zone 5 and its mirror zone, ETDRS zone 3.

cysts and Figure 2 for 3-D false colour views. In the 28 study eyes with an intraretinal cyst at baseline, 9 disappeared or considerably reduced. For fellow eyes, 9 initially showed a cyst but this increased to 20 at the end of the study.

There were also significant changes in the retinal thickness, measured by OCT as shown in Table 1.

We also calculated the change in the various retinal locations of the study eyes only (Table 2). Only the reduction of thickness in the zone of maximal oedema is significant. The change in thickness of the mirror zones is negligible. Table 2 also gives the changes in dimension for the entire study (months 1–6), and the first half (months 1–3) and second half (months 3–6). There were no significant changes in the fellow eyes (not illustrated).

We also calculated the quantity (change in thickness in study eye)–(change in thickness of its fellow eye) for each of the three zones indicated in Table 1. The difference was significant for central and maximal zones, but not for the mirror zone (Table 3).

Figure 3 shows distribution of the individual differences. For fellow eyes, swelling increases in 16 and

Table 1 Measurements of retinal thickness in the study and fellow eyes

Location	*Study eyes* n = 34				*Fellow eyes* n = 34				*Change in thickness (negative = reduction of thickness) 95% CI after 6 months*
	Thickness at baseline (μM)		*Thickness after 6 months (μM)*		*Thickness at baseline (μM)*		*Thickness after 6 months (μM)*		
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	
Average of cube	283	20	282	20	282	23	282	23	−13 to +8
ETDRS zone 1	277	55	265	55	249	38	248	40	−25 to +0.8
ETDRS zone with maximal thickness	343	48	322	26	333	51	328	39	−18 to +8
Opposite (mirror) zone of the maximal thickness zone in the study eye	318	18	318	20	312	26	310	27	−5.4 to 2.4

Measurements of retinal thickness (outer limiting membrane to retinal pigment epithelium) for the average of the region scanned and three separate zones, corresponding to the ETDRS zones. Note that the average retinal thickness was similar (and within normal limits [3]) for trial and fellow eyes. For the central and the most swollen zones, the study eyes improved, but the fellow eyes' corresponding zones did not. For the mirror zones, there was little difference between study and fellow eyes.

Table 2 Mean change in retinal thickness for various OCT zones in the study eyes

	Central subfield zone 1			*Zone of maximal oedema*			*Opposite (mirror) zone of the maximal thickness zone in the study eye*		
Month	1–3	1–6	3–6	1–3	1–6	3–6	1–3	1–6	3–6
Mean	6.3	5.9	7.7	9.1	12.0	7.6	0.26	0.04	−0.25
SD	28.9	34.2	47.8	21.9	23.8	22.7	17.9	15.0	15.2
SE	5.37	6.6	9.2	4.2	4.6	4.4	3.5	2.9	2.9
t-test	0.13	0.18	0.20	**0.01**	**0.01**	**0.04**	0.47	0.50	0.47

Means and statistics of reduction of study eyes' retinal thickness, for various OCT zones, between baseline and the end of the study. *t*-test gives the probability that the observed change is not different to zero. Significant results are emboldened ($n = 34$ eyes). The 95% confidence intervals can be obtained by adding or subtracting $2.045 \times$ SE from the means.

Table 3 Each fellow eye is treated as control for its study eye

(Change in study eye) − (Change in fellow eye) (μm)				
Zone	*Mean*	*SE*	*95% CI*	P-*value*
Central	−11	1.92	−14.7…−.6.9	0.026
Maximal	−13	2.26	−17.5…−7.2.	0.052
Mirror	2.4	2.41	−2.5…7.3	0.144

Each fellow eye is treated as control for its study eye The retinal thickness in study eyes shrinks to a greater extent than in the fellow eye for the central zone and the zone of maximal swelling but only reaches significance for the central zone. The increase in thickness of the mirror zone is insignificant.

decreases in 18. For study eyes, swelling increases in 8 and decreases in 26.

Psychophysical testing and achromatic contrast sensitivity testing

The average thresholds of illuminated and fellow eyes at baseline were not significantly different (*t*-test: $P = 0.39$ for acuity and 0.214 for contrast sensitivity) but after 6 months the difference between study and fellow eyes had increased ($P = 0.001$ for acuity and 0.054 for contrast sensitivity). Note that the average final visual acuity of >80 letters read corresponds to a value slightly better than 20/20.

Of the 13 patients initially having BCVA < 80 in the study eyes, 10 improved by 5 letters or more. For fellow eyes, corresponding numbers were 9 of whom 3 improved, 6 remain unchanged, while 3 deteriorate by >3 letters (Fisher exact test gives $P = 0.054$). Contrast sensitivity increased by 3 or more letters on Pelli–Robson charts in 11 trial eyes, remained unchanged in 6, while 3 deteriorate by more than 3 letters. In only 1 of the 9 patients with an initial score of <28 letters was there deterioration (Fisher exact $P = 0.003$). The results are shown in Table 4.

Table 5 summarises the results from microperimetry. Patients found it difficult to carry out the test, and only 25 complete results were obtained 3 months, and the test was then abandoned. The sensitivity of the central area and the peripheral zones of treated eyes increased while it decreased in the untreated eyes.

Discussion

Synopsis of results

This relatively small study has provided evidence that in patients with very early DMO, significant local

morphological and functional improvements occur in the eye exposed to light during sleep. In untreated eyes, any morphological change is a deterioration.

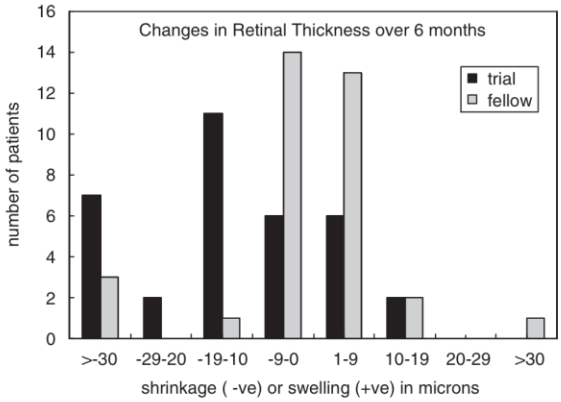

Figure 3 Magnitude of reduction in retinal thickness of the worse affected ETDRS zone of treated and fellow eyes, where swelling was maximal. This zone (2,3,4, or 5) was determined by the initial measurement and subsequent shrinkage or swelling was determined for that zone. The zone number differed from patient to patient. The fellow eye results are distributed equally around zero, but the changes in the study eyes indicate that there was considerable more shrinkage than swelling. Note such results are not distributed in a Gaussian manner. See Discussion for a possible explanation.

Possible explanations

The mean OCT changes are however small, because we confined recruitment to the earliest stage of the condition. In such patients, local regions of oedema may disappear but the general course of the disease is a progression, and as one region of leakage reduces others may take its place. This cannot explain our results in the study eyes: the 'mirror zones' did not change. Standard statistical methods thus show that the changes associated with treatment are unlikely to be due to chance. We have not included in the analysis the results of the four patients withdrawn from the study because only the unilluminated eyes required lasering during the study period. This also underestimates the significance of the results. In psychophysical tests, the average magnitude of the improvement was small. But a high proportion of our patients had normal or nearly normal acuity at study onset, so the changes are subject to the 'ceiling effect': in those with initially reduced acuity, 10 of 13 showed improvement of >5 letters. Most of the improvement occurred in the first 3 months of the study, though often return to normality was observed in the latter half of the short study. By contrast, there was deterioration of these other measures of function in untreated eyes.

Study limitations and failings

We have not conducted a double-masked randomised study. This is partly due to lack of resources. The

Table 4 Changes in psychophysical tests

Test	*Study eyes*				*Fellow eyes*				*Change and 95% CI after 6 months*
	Initial values		*Final values*		*Initial values*		*Final values*		
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	
Visual acuity (ETDRS letters)	78.04	7.80	80.18	5.32	76.52	8.10	74.59	8.52	4.21
	Range 85–60		Range 85–60		Range 86–67		Range 87–47		2.64–5.78
Constrast sensitivity (Pelli–Robson) letters read	31.71	5.39	33.14	4.57	30.16	4.71	30.50	4.95	2.00
	Range 38–20		Range 41–20		Range 36–23		Range 36–20		1.25–2.75

Summary of results of visual acuity and contrast sensitivity testing in study and fellow eyes.

Table 5 Summary of microperimetry results in trial eyes for first 3 months of trial

	Microperimetry results: mean retinal sensitivity in dB					
	Means ± SD: n = *25*					
	Zone 1		*Mean of zones 2–9*		*Change 0–3 months*	
	Initially	*3 months*	*Initially*	*3 months*	*Zone 1*	*Zone 2–9*
Study eye	14.13 ± 3.42	14.86 ± 3.43	14.85 ± 2.47	15.67 ± 2.44	−0.94	−0.97
Fellow eye	14.46 ± 4.17	12.58 ± 5.00	14.55 ± 3.60	13.33 ± 4.75	1.13	0.54
t-test (study–fellow)					$P = \mathbf{0.0027}$	$P = \mathbf{0.0021}$

Significant results are shown in bold.

light masks had a high failure rate so some patients did not have continuous periods of illumination at night. We had no means of determining whether the light-emitting diodes were always positioned in front of the pupil, or if they could become displaced during the night. The magnitude of change in individual cases (Figure 3) is not distributed normally and this could be accounted for if adequate treatment was not given in some cases. However, these failings in the mask would reduce the efficiency of the treatment, and increase the significance of any improvement associated with the use of the masks. We have measured local changes in retinal thickness to demonstrate changes in the oedema, a practice which is not 'standard' but has been previously employed.[27] As in two respects—number of cysts and thickness of the ETDRS Zone 1—study and fellow eyes were not equal, it is not certain whether the fellow eyes could be used as controls for the study eyes. We have therefore carried out both inter-eye comparisons, comparison between pairs of eyes in the same individuals, and longitudinal comparisons on study eyes only. All methods of analysis indicate that the observed improvements were significant.

Comparison with other trials

Most previous trials of treatment have been designed to test the efficacy of invasive methods, and therefore treatment has been given to patients with more severe retinal changes than the patients in this study. Direct comparison of our results with other trials is therefore not possible, and our results cannot be directly used to estimate the value of the treatment for patients with more advanced disease.

Implications for the pathophysiology of DR

The lids and the media attenuate light from the mask by a factor of ≈ 100.[28,29] If all the electricity used was converted to photons, the retinal illumination would be $\approx 1\,\mu W/cm^2$. Such a light level cannot cause damage to retinal structures, or significant heating, or affect the function of mitochondria. Retinal ganglion cells containing melanopsin[30] respond directly to light and this has been linked with visual disturbances[31–33] and circadian rhythm change associated with melatonin. The retinal illumination (480 nm) required to cause a 50% reduction in the night-time increase of melatonin is $25\,\mu W/cm^2$.[34,35] The trans-lid illumination caused by the masks would therefore produce minimal changes in melatonin. The only known way for such weak light to reverse retinal changes is thus via the hypothesis already advanced,[21] that during dark adaptation, rod metabolic activity increases and the resulting hypoxia stimulates the production of cytokines that cause retinal damage. Thus the study may be considered a proof of principle.

Limitations of the study

We have not encountered any adverse effect, but the study was short. Any adverse effect on the retina of low intensity, continuous illumination is most unlikely, and might even improve retinal function, if it inhibited the synchronous shedding of rod tips that occurs each dawn.[34] If rods 'shed' individually throughout the day, the ability of RPE cells to phagocytose and digest rod discs might improve. In rodents, which are very susceptible to light damage, increasing the average 24 h illumination within tolerable limits only results in a shortening of the rod outer limb, which is autoregulatory, and results in the cell absorbing a constant 14 000 photons/rod/second.[34–38] We calculate that the illumination of the light masks provided only 50–500 photons absorbed/rod/sec.

Generalisability

Evidently, large-scale fully randomised and controlled clinical trials are required to establish the efficacy of this method of treatment, the quantity of light required, the long-term effects, if any, the stages in DR for which such interventions improve retinal function, and how long any improvement can be maintained.

Possible significance for DR

Providing light during sleep is inexpensive and methods of obtaining it are readily available in places where there is a constant electricity supply. The use of light masks similar to those we have made has certain advantages: the intensity of light is known, and not subject to the patient covering the eyes. The cost of running the mask compares favourably with the cost of electrical room illumination. The provision of light at night, from whatever source, may not require supervision because the non-invasive treatment has no known adverse effects. Therefore, even if the method only functions to reverse the earliest retinal diabetic changes, it might be widely adopted by people with diabetes as a prophylactic measure. However, this study does not provide any evidence that light therapy would be helpful in proliferative DR or more advanced cases of non-proliferative DR, but it might delay the onset of more serious effects of DR.

Summary

What was known before

- Dark adaptation requires significant oxygen demand. VEGF is increased in diabetic macular oedema and the main stimulus for it is hypoxia.

What this study adds

- Trans-eyelid retina illumination causes regression of diabetic macular oedema due to decrease in dark adaptation-associated oxygen consumption.

Conflict of interest

The authors declare no conflict of interest.

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